JOURNAL OF AGRICULTURAL RESEARCH

VOL. 62 WASHINGTON, D. C., APRIL 1, 1941 No. 7

STERILITY AND ABERRANT CHROMOSOME NUMBERS IN CALORO AND OTHER VARIETIES OF RICE [1]

By JENKIN W. JONES, *senior agronomist*, and A. E. LONGLEY, *associate botanist*, *Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture*

INTRODUCTION

Recent interest in the artificial production of polyploids in various plant species suggested the desirability of a search for similar but naturally occurring chromosomal aberrants in rice. Most of these were sought in the Caloro variety growing at the Biggs Rice Field Station, Biggs, Calif.

In this rice-growing section maximum daily temperatures are high, and the daily range of temperature is often as much as 40° F. during the summer months. Extremes and sudden changes in temperature have been reported to cause chromosomal aberrations in plants, and Matusima (*12*) [2] reported the finding of tetraploid cells in the root tips of rice seedlings exposed for a short time to temperatures of 42° to 45.5° C.

The stage at which chromosomes are most easily affected by environmental changes is thought to be at the time of sporogenesis. In rice this appears to occur 10 to 15 days prior to heading. In the Caloro variety in California it occurs during and following periods of high temperatures and wide daily ranges in temperature. For example, in 1935, 1936, and 1937, the maximum temperature varied from 100° to 108° F. and the daily range was as high as 47° during the period in which the Caloro variety was forming gametes. It is possible, therefore, that such extremes and ranges in temperature may be responsible in part for the chromosome aberrations reported in this paper. The temperature may act directly upon meiosis, resulting in the production of abnormal gametes similar to those observed by Nandi (*29*), or it may cause somatic mutations that eventually embrace the germ cells.

Most of the material on which cytological studies were made was collected by the senior writer at the Biggs Rice Field Station, and all cytological studies were made by the junior writer.

The Caloro variety, in which most of the material was collected, is the leading commercial rice in California. It was selected at Biggs in 1913 by E. L. Adams [3] and C. E. Chambliss [3] from Early Wataribune, a variety of rice (*Oryza sativa* L.) introduced from Japan in the same year by W. K. Brown, of Butte City, Calif. Caloro was distributed and named by the senior writer in 1921. It is a midseason, short-grain variety.

[1] Received for publication December 2, 1940.
[2] Italic numbers in parentheses refer to Literature Cited, p. 396.
[3] Of the Bureau of Plant Industry.

PREVIOUS INVESTIGATIONS

Prior to 1930, all the cultivated varieties and mutants of rice that had been investigated cytologically had 12 chromosomes in their pollen mother cells. In the last 10 years, however, haploid, aneuploid, triploid, and tetraploid plants also have been discovered and described. In the following tabulation is given a list of the papers published on cytological studies of *Oryza sativa*.

Somatic chromosome number:	References
Diploid 24	Kuwada (*11*) 1910; Nakatomi (*27*) 1923; Rau (*50*) 1929; Kato, Kosaka, Hara, Maruyama, and Takiguchi (*10*) 1930; Selim (*52*) 1930; Morinaga and Fukushima (*16*) 1931; Nakamura (*25*) 1931; Ramiah (*42*) 1931; Hedayetullah (*3*) [1934]; Ichijima (*9*) 1934; Ramiah, Parthasarathi, and Ramanujam (*48*) 1935; Sakai (*55*) 1935; Miyazawa (*13*) 1935; Ramanujam (*36*) 1935, (*37*) 1936, (*40*) 1938; Nandi (*28*) 1936, (*29*) 1937; Takahashi (*54*) 1936; Sethi (*53*) 1937; Parthasarathy (*34*) 1938, (*35*) 1939.
Aneuploids 24+1 or more	Nakamori (*23*) 1932; Ichijima (*9*) 1934; Ramanujam (*38*) 1937; Parthasarathy (*33*) 1938.
Haploid 12	Morinaga and Fukushima (*17*) 1931, (*18*) 1932, (*19*) 1934; Ramiah, Parthasarathi, and Ramanujam (*45*) 1933, (*47*) 1934; Nakamura (*26*) 1933; Ichijima (*9*) 1934; Takahashi (*54*) 1936.
Triploid 36	Nakamori (*23*) 1932; Ramiah, Parthasarathi, and Ramanujam (*46*) 1933; Ichijima (*9*) 1934; Morinaga and Fukushima (*20*) 1935; Ramanujam (*38*) 1937.
Tetraploid 48	Nakamori (*24*) 1933; Ichijima (*9*) 1934; Morinaga and Fukushima (*21*) 1936, (*22*) 1937.

HAPLOID PLANTS

In 1931, Morinaga and Fukushima (*17*) found a rice plant among F_1 plants of an intervarietal cross between Dekiyama (normal) and Bunkitu-to, a dwarf variety, that proved to be a haploid. In 1933 Nakamura (*26*), and Ramiah, Parthasarathi, and Ramanujam (*45*) reported on haploid rice plants, and since then others have been reporting on similar studies (see tabulation above). A small sterile F_1 plant from the cross Blue Rose × Carolina Gold was observed at the Biggs Rice Field Station in 1930. This plant was shorter and had narrower leaves, shorter panicles, and much smaller florets than other F_1 plants of the same cross; it was morphologically essentially the same as plants that later proved to be haploids. The collection of verified haploid plants of rice in the United States began in 1934. In that year, H. M. Beachell [4] sent to Washington, D. C., a small sterile plant from a segregating population of a cross between Rexoro × Blue Rose, and in 1936 a similar plant from the Latex variety. In 1936, N. E. Jodon [5] also sent to Washington a small sterile plant from C. I.[6] 81C × Blue Rose, and the senior writer sent in a similar plant from a pure-line selection (No. 241B7–29) from the Biggs Rice Field Station. These four plants have been

[4] Of the Division of Cereal Crops and Diseases, stationed at Texas Agricultural Substation No. 4, Beaumont, Tex.
[5] Of the Division of Cereal Crops and Diseases, stationed at the Rice Experiment Station, Crowley, La.
[6] C. I. refers to accession number of Division of Cereal Crops and Diseases.

grown in a greenhouse since they were received from the field, and in 1937, 12 univalent chromosomes were found in the prophases of the first reduction division of their pollen mother cells.

These haploid plants grow more slowly and have smaller culms and narrower leaves than normal plants of the varieties or populations from which they arose, and the panicle branches are short and the florets are small and sterile. Haploid No. 241B7–29 often produces a relatively large number of poorly developed parthenocarpic seeds, none of them being viable. The senior writer, with L. L. Davis,[7] found 11 haploidlike plants among the varieties, segregating populations, hybrid selections, and backcrosses growing on an area of less

FIGURE 1.—First-division metaphase chromosomes of a pollen mother cell of a haploid mutant (Latex) of *Oryza sativa*. × 3,000.

than 1 acre and comprising not less than 300,000 plants at the Biggs Rice Field Station in 1939. Two of these were in the Caloro variety. Since 1934, haploid plants also have been observed from time to time at the rice experiment stations in the Southern States.

The behavior of the chromosomes of haploid plants during meioses has been described and illustrated by Morinaga and Fukushima (*18, 19*), Ramiah, Parthasarathi, and Ramanujam (*45, 47*), and others.

The studies reported here confirm the cytological findings of investigators in Japan and India. It was determined that in the four haploid plants studied the number of bivalent chromosomes at first metaphase never exceeded two (fig. 1); that the synapsis of the bivalents was incomplete; and that split univalents occasionally occurred in first anaphases.

[7] Of the Division of Cereal Crops and Diseases.

STERILE DIPLOID PLANTS

Single culms of 72 sterile and partly sterile Caloro plants were collected from plots at the Biggs Rice Field Station on October 13, 1938. These plants were fully headed but greener than normal mature plants in the same plots. Sterile and partly sterile rice plants usually produce additional late tillers early in the fall, apparently utilizing food materials normally required for the developing grain. Such plants can be detected readily in a mature field of rice. Some of the plants collected in 1938 had larger florets than Caloro; in others there was no appreciable difference from normal plants in floret or seed size but some difference in shape. Plants with larger florets than Caloro were highly sterile but produced some parthenocarpic seed. This sort of development was less common, but not absent, in all the partly sterile plants with normal floret size, which ranged in fertility from 0.91 to 79.25 percent.

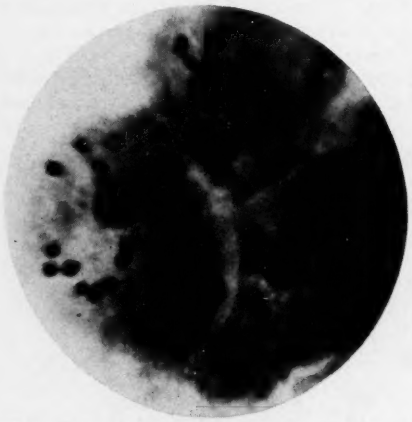

FIGURE 2.—Photomicrograph showing the 12 bivalent chromosomes in pollen mother cells of *Oryza sativa*, variety Caloro. × 800.

The culms collected were sent to Washington, D. C., and were transplanted in pots in a greenhouse at the Arlington Experiment Farm, Arlington, Va. In the spring of 1939 panicles were collected for cytological studies.

As was expected, most of the plants examined proved to be diploid. Twenty-one of the plants showed a normal chromosome behavior (fig. 2) and the first-division anaphases gave excellent figures for determining their chromosome numbers (fig. 3). The reduction phases of these plants did not differ from those of normal diploid rice varieties described and illustrated by Kuwada (*11*), Nakatomi (*27*), Rau (*50*), Kato et al. (*10*), and others.

FIGURE 3.—A first-division anaphase from a pollen mother cell of a diploid plant of *Oryza sativa*, variety Caloro. × 3,000.

Certain abnormalities in the pollen mother cells and in the behavior of the chromosomes during meioses were observed in 14 diploid plants. In four of these plants there was a tendency for partial asynapsis of from two to many of the chromosomes, somewhat similar to the condition reported by Ramanujam and Parthasarathy (*41*). One plant showed very few bivalent chromosomes at first metaphase, a second frequently had as many as 12 univalents at metaphase (fig. 4),

and the remaining two plants generally showed a few unpaired chromosomes in the early phases of the first division. In two of these plants there were no normal anthers. Many of them had been transformed into normal-appearing pistils, and the few anthers present contained very little sporophytic tissue.

Poorly developed sporophytic tissue was characteristic of all the 14 plants that showed abnormal pollen mother cells. There were

FIGURE 4.—A first-division metaphase from a pollen mother cell of a sterile diploid plant of *Oryza sativa*, variety Caloro. × 3,000.

few pollen mother cells, and consequently it was hard to find any cells undergoing division. The mother cells in these plants varied greatly in size and in their development failed to show the uniformity characteristic of diploid plants. These conditions limited the studies of chromosomes to the more normal cells. In all 14 plants, the more normal cells showed 24 chromosomes on the first-division anaphase spindle.

Miyazawa (*13*) described the genetic behavior and gave the chromosome numbers for several types of sterile mutants of rice. Neither in his material nor in these 14 plants was there a change in the chromosome number that might account for the pollen sterility. It is possible, however, that an explanation of the meiotic irregularities observed might be found by a more detailed morphological study of the individual chromosomes.

ANEUPLOID PLANTS

Nakamori (*23*) first reported aneuploid rice plants, and Ichijima (*9*) reported two heteroploid plants, each with an extra chromosome from progeny of artificially treated material. Parthasarathy (*33*) also reported on aneuploids, and Ramanujam (*38*) recently reported on forms having $2n=24, 25, 26, 27, 28, 29,$ and 30 chromosomes. One panicle was gathered from each of several highly sterile Caloro plants growing in plots at the Biggs Rice Field Station on October 9, 1937. The spikelets of these plants were distinctly larger than those of normal Caloro. The florets of the panicles were sterile, except for an occasional seed and a few poorly developed parthenocarpic seeds that failed to germinate. A few plants, however, were obtained from these seeds, and the pollen mother cells of five of these plants were investigated in 1938.

FIGURE 5.—A first-division metaphase from a pollen mother cell of an aneuploid plant ($2n+1$) of *Oryza sativa*, variety Caloro. × 3,000.

Of the five plants studied, three had $2n+2$ and two had $2n+1$ chromosomes. The presence of one or more extra chromosomes was often indicated in the prophases of the first division when they showed more than the usual 12 chromosome group characteristics of the true diploid. At metaphase the presence of extra chromosomes in the aneuploid plants was apparent, since at this stage bivalents are readily distinguished from univalents (fig. 5). It was the anaphase, however, that proved to be most useful for making unquestionable counts (fig. 6). Three of these aneuploid plants had 26 chromosomes on the anaphase spindle and two of them 25.

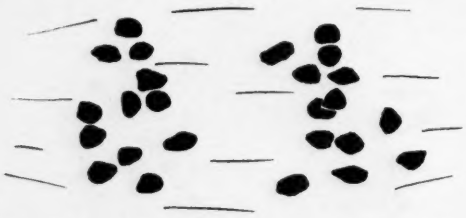

FIGURE 6.—A first-division anaphase from a pollen mother cell of an aneuploid plant ($2n+2$) of *Oryza sativa*, variety Caloro. × 3,000.

Lack of time and an insufficient number of pollen mother cells from these plants prevented as complete a study of the chromosomes of this group as they deserved.

These aneuploid plants probably originated as crosses between triploid mutants, or partially sterile diploids that produce some gametes with one or more extra chromosomes, and diploid plants growing in the same plots. Ramanujam (*38*) has reported on trisomics in rice that originated in this manner. Triploid plants are apparently fairly common in the Caloro variety at Biggs, Calif., and it probably would not be very difficult to select fromp lants obtained from seed of triploids × diploids the 12 primary trisomic types. These could then be used in linkage studies.

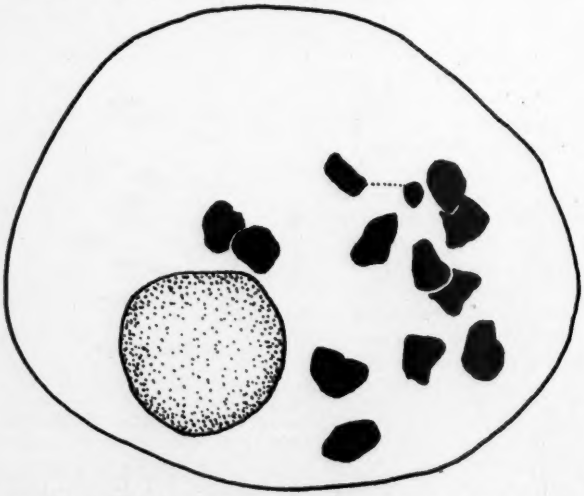

FIGURE 7.—Diakinesis from a pollen mother cell of a triploid plant of *Oryza sativa*, variety Caloro. × 3,000.

TRIPLOID PLANTS

Soon after the discovery of a haploid rice plant, Nakamori (*23*) and Ramiah, Parthasarathi, and Ramanujam (*46*) reported on triploid plants. Later, Ichijima (*9*) found 11 triploids among a group of artificially induced mutations; and Morinaga and Fukushima (*20*) found triploid plants in considerable numbers in commercial fields.

The chromosomes of the somatic cells, as well as the meiotic phases of triploids, have been illustrated and described by Nakamori (*23*), Ichijima (*9*), Morinaga and Fukushima (*20*), and Ramanujam (*38*). Meioses have been so well described by Morinaga and Fukushima (*20*) that there seems to be little more to add from this study except to stress the difficulty of detecting in the early first-division phases the triploid nature of plants.

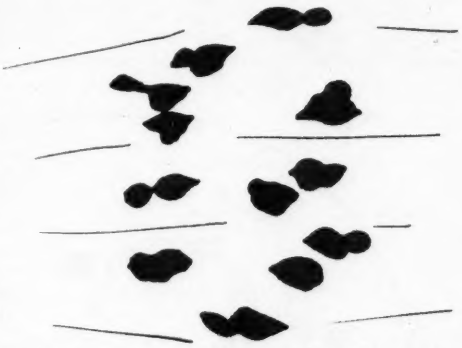

FIGURE 8.—A first-division metaphase from a pollen mother cell of a triploid plant of *Oryza sativa*, variety Caloro. × 3,000.

Nine plants in the Caloro collection of 1938 had the $2n$ chromosome number 36. In prophases the finding of 12-chromosome groups that showed very little indication of their trivalent nature was the rule (fig. 7).

The trivalent nature of the chromosomes became more apparent at metaphase. At this phase a trivalent often looked like a bivalent with a univalent loosely attached (fig. 8). In some cells at metaphase,

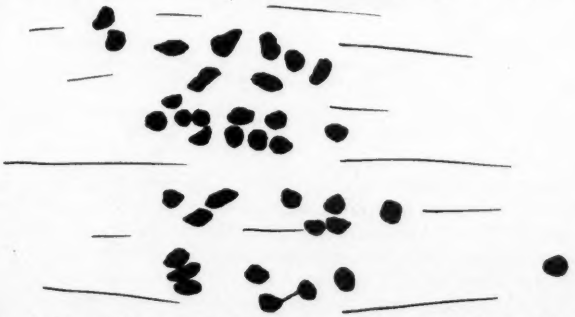

FIGURE 9.—A first-division anaphase from a pollen mother cell of a triploid plant of *Oryza sativa*, variety Caloro. × 3,000.

univalents, bivalents, and trivalents were present and distinguishable from one another. However, it was the first-division anaphase that showed all 36 chromosomes distinctly separated on the spindle (fig. 9).

Nine other plants in the same collection were highly sterile in 1938 and had large florets similar to the plants that proved to be triploids, so they, too, apparently were triploids. Both a sterile Early Prolific

plant and a sterile selection in the ninth generations of the cross Colusa × Blue Rose had larger florets than the parent varieties at Biggs, Calif., in 1938, and proved to be triploids. In the fall of 1939 several highly sterile plants, similar to the triploids collected in 1938, were again observed in plots of Caloro at the Biggs Rice Field Station.

TETRAPLOID PLANTS

Nakamori (*24*), Ichijima (*9*), and Morinaga and Fukushima (*21*) described tetraploid plants of *Oryza sativa* and illustrated both the somatic chromosomes and the chromosomes at meioses.

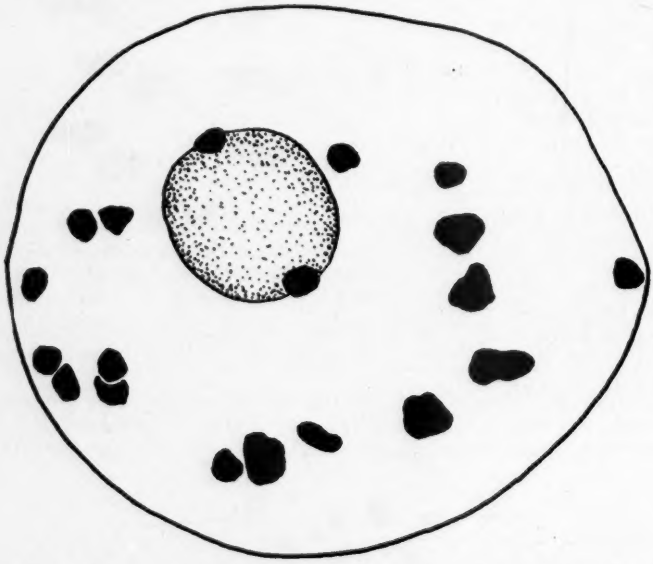

FIGURE 10.—Diakinesis from a pollen mother cell of a tetraploid plant of *Oryza sativa*, variety Caloro. × 3,000.

One plant in the 1938 collection of Caloro proved to be a tetraploid. The meiotic behavior in pollen mother cells from this plant was found to be very similar to that described by Morinaga and Fukushima (*21*). The prophase showed the chromosomes in groups or clumps that differed considerably in size (fig. 10). These groups gave little or no clue, other than their size, to the number of chromosomes they contained. At metaphase, however, the figures had changed; bivalents were most frequent, but in most plates several tetravalent groups were present, looking very much like two bivalents lying side by side (fig. 11).

Trivalents and univalents were exceptional in the early meiotic phases of this tetraploid, and consequently at anaphase the 48 chromosomes usually were equally divided and moved to the poles in a regular manner (fig. 12). Abnormalities in the later phases of meioses were of infrequent occurrence, and a regular pollen tetrad was characteristic.

In 1939, another tetraploid Caloro plant collected at Biggs, Calif., had larger seeds and longer awns than normal Caloro. The single panicle on this plant, which was growing on infertile soil, produced only 35 seeds and 13 sterile florets; it was therefore 27.1 percent sterile. This plant was similar in all morphological characters to the tetraploid plant reported above, except that the panicle examined had a higher degree of fertility. This probably was due to the fact that a panicle from a tiller was saved from the plant collected in 1938, whereas the panicle of the main culm was collected in 1939.

FIGURE 11.—A first-division metaphase from a pollen mother cell of a tetraploid plant of *Oryza sativa*, variety Caloro. X 3,000.

CELL SIZE IN HAPLOID, DIPLOID, TRIPLOID, AND TETRAPLOID CALORO PLANTS

A study was made of the relative size of the pollen and the guard cells of the stomata in haploid, diploid, triploid, and tetraploid plants, all from the Caloro variety of rice.

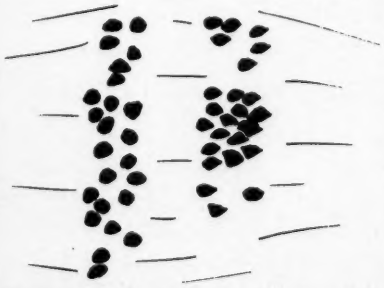

FIGURE 12.—A first-division anaphase from a pollen mother cell of a tetraploid plant of *Oryza sativa*, variety Caloro. × 3,000.

Pollen of the four types showed a progressive increase in size from the haploid to the tetraploid, but since there is considerable variation in pollen size in the haploid, triploid, and tetraploid plants, it is believed that the guard cells are more useful for determining the comparative cell sizes. The increase in cell size may be seen in figure 13, *A*, *B*, *C*, and *D*, which shows the guard cells and a few of the adjoining cells from haploid, diploid, triploid, and tetraploid plants, respectively, that differ in chromosome numbers by multiples of 12.

In the spring of 1939, diploid, triploid, and tetraploid Caloro plants were subdivided and transplanted, on the same date, in a cage at Arlington Experiment Farm, Arlington, Va. The plants were spaced

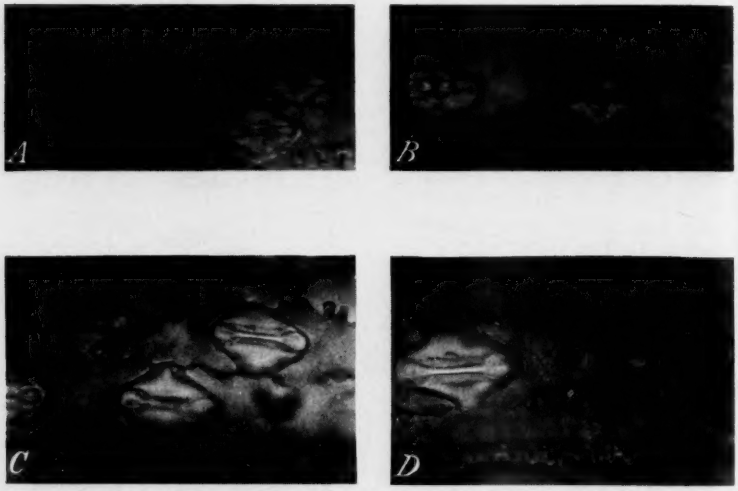

FIGURE 13.—Photomicrographs showing the relative size of the guard cells from four plants of *Oryza sativa*, variety Caloro: *A*, Haploid; *B*, diploid *C*, triploid; *D*, tetraploid. × 350.

about 10 inches apart in rows spaced 1 foot apart and were grown on submerged land under field conditions. Some of the characteristics of these plants are shown in table 1.

TABLE 1.—*Characteristics of diploid, triploid, and tetraploid Caloro plants grown at Arlington Experiment Farm, Arlington, Va., 1939*

Type	Plants	Average				
		Leaf width		Culms per plant having panicles	Height	Panicle length [1]
		Flag	First leaf below flag			
	Number	*Mm.*	*Mm.*	*Number*	*Cm.*	*Cm.*
Diploid	5	13.4	17.2	15.8	111.8	22.8
Triploid	5	14.3	17.5	4.8	110.7	23.6
Tetraploid	8	12.2	15.9	1.6	95.2	17.0

[1] Measured from tip to basal node of panicle.

The meager data presented in table 1 show that the diploid plants were taller and produced many more productive tillers and longer panicles than the tetraploid plants. The triploid plants were nearly

as tall as the diploid; they tillered less but produced somewhat longer panicles. The tetraploid plants had narrower flag leaves than the

FIGURE 14.—Panicles of haploid (*A*), diploid (*B*), triploid (*C*), and tetraploid (*D*) Caloro plants grown at the Arlington Experiment Farm, Arlington, Va.

diploid or triploid plants. The tetraploid plants were distinctly coarser in all morphological characters and less vigorous and productive than the diploid plants. These polyploid forms appear to be of no practical value (figs. 14 and 15).

FIGURE 15.—Panicles of haploid (*A*), diploid (*B*), triploid (*C*), and tetraploid (*D*) Caloro plants grown at the Biggs Rice Field Station, Biggs, Calif., in 1939.

CYTOLOGY OF WILD RICE BELONGING TO ORYZA AND OTHER GENERA

Cytological studies on rice have not been entirely restricted to the cultivated species, *(Oryza sativa)*, and a chronological summary of the reported results of chromosome studies in the genus *Oryza* and other genera that have a similar habitat should be of interest and are, therefore, shown in table 2.

TABLE 2.—*Chronological list of papers published on cytological studies of wild rice (Oryza) and other genera*

Species	Somatic chromosomes	Reference
	Number	
Oryza cubensis Ekman	24	Gotoh and Okura (*1*) 1933.
O. formosana Masamune and Suzuki	24	Do.
O. latifolia Desv. (Cuba)	48	Do.
O. latifolia Desv. (Asia)	24	Ramiah (*43*) 1934; Heyn (*4, 5*) 1936, 1938.
O. minuta Presl	48	Morinaga (*14*) 1934; Ramiah (*44*) 1936; Nandi (*28*) 1936; Ramanujam (*40*) 1938.
O. sublata Nees	24	Horovitz and Pogliaga (*8*) 1934.
O. longistaminata A. Chev. and Roehr	24	Ramiah et al. (*49*) 1935; Ramanujam (*40*) 1938.
Do.	48	Ramiah et al. (*49*) 1935.
O. officinalis Wall.	24	Nandi (*28*) 1936.
O. barthii A. Chev.	24	Heyn (*4, 5*) 1936, 1938; Ramanujam (*40*) 1938.
O. meyeriana Baill.	24	Heyn (*4, 5*) 1936, 1938.
O. sativa var. *spontaneae*	24	Do.
O. glaberrima Steud.	24	Ramanujam (*40*) 1938.
O. coarctata Roxb.	48	Parthasarathy (*34*) 1938.
Zizania aquatica L.	30	Nandi (*28*) 1936; Longley[1] 1938; Ramanujam (*40*) 1938.
Z. latifolia Turch.	34	Hirayoshi (*7*) 1937.
Do.	30	Ramanujam (*40*) 1938.
Leersia japonica Hack.	60	Hirayoshi (*7*) 1937.
L. japonica Makino	96	Do.
L. oryzoides Sw.	48	Ramanujam (*40*) 1938.
L. hexandra Sw.	48	Hirayoshi (*7*) 1937; Ramanujam (*40*) 1938.
Hygroryza aristata Nees	24	Do.
Chikusichloa aquatica Koidz.	24	Hirayoshi (*7*) 1937.
Ligeum spartum L.	40	Ramanujam (*40*) 1938.

[1] Unpublished data.

The only chromosome determinations made in this study, other than on forms of *Oryza sativa*, were from the pollen mother cells of *O. latifolia* Desv. from Cuba and of the same species from the Botanical Gardens of the Straits Settlements, British Malaya. The specimen

FIGURE 16.—A first-division anaphase from a pollen mother cell of a diploid form of *Oryza latifolia*. × 3,000.

studied of *O. latifolia* from Cuba has the tetraploid chromosome number 48, as reported also by Gotoh and Okura (*1*). *O. latifolia* from the Straits Settlements has the diploid chromosome number 24 (fig. 16), as previously reported by Ramiah (*44*) and Heyn (*4, 5*).

The chromosomes of the tetraploid plant of *Oryza latifolia* were all bivalents at the first-division prophases (fig. 17), and the later meiotic

phases showed a regular distribution of 24 chromosomes to each of the four cells of the pollen tetrad.

Plants of the tetraploid form of *Oryza latifolia* (from Cuba) are much more vigorous and have larger culms, leaves, panicles, and somewhat larger seeds than the diploid form from Asia.

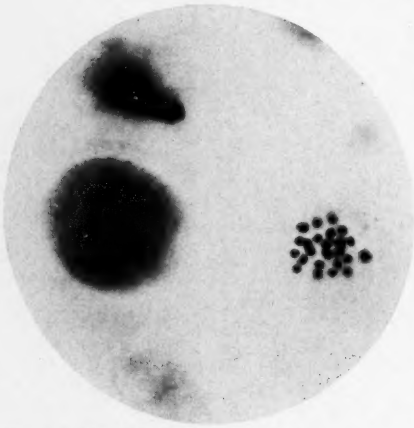

FIGURE 17.—Photomicrograph showing metaphases in the pollen mother cells of a tetraploid form of *Oryza latifolia*. × 500.

INTERSPECIFIC HYBRIDS IN RICE

In 1931, Ramiah (*42*) reported attempts to cross *Oryza sativa* with the wild (diploid 24) species *O. longistaminata* A. Chev. and Roehr. and *O. latifolia* (from Asia), but only parthenocarpic seed were obtained. Later, however, Ramiah (*44*) reported that a successful cross had been made between *O. sativa* and *O. latifolia* (from Asia). A chronological list of the interspecific crosses reported with *Oryza* is shown in table 3. Gotoh and Okura (*2*) reported on crosses of *O. sativa* × *O. cubensis* Ekman, and *O. sativa* × *O. latifolia* (from Cuba). The senior writer also made the cross Colusa (*O. sativa*) × *O. cubensis* in 1933; the F_1 plants were sterile and more like *cubensis* than Colusa. This agrees with the observations of Gotoh and Okura (*2*). The F_1 plants were backcrossed to Blue Rose (*O. sativa*), and from this progeny and progeny from natural crosses with *O. sativa* some lines rather resistant to *Helminthosporium oryzae* Van Breda de Haan have been isolated. Interspecific crosses in rice appear to be easier to make when *O. sativa* is used as the female parent. Morinaga and Aoki (*15*) have also shown that certain cultivated varieties cross more readily with wild rice than do others.

TABLE 3.—*Chronological list of reported interspecific crosses in Oryza*

Cross	Somatic chromosomes	Reference
	Number	
O. sativa × *latifolia* (Asia)	24×24	Ramiah (*43*) 1934.
O. sativa × *latifolia* (Cuba)	24×48	Gotoh and Okura (*2*) 1935; Hirayoshi (*6*) 1937; Morinaga and Aoki (*15*) 1938.
O. latifolia (Cuba) × *sativa*	48×24	Morinaga and Aoki (*15*) 1938.
O. sativa × *cubensis*	24×24	Gotoh and Okura (*2*) 1935.
O. sativa × *minuta*	24×48	Morinaga (*14*) 1934; Okura (*32*) 1937; Morinaga and Aoki (*15*) 1938.
O. minuta × *sativa*	48×24	Nandi (*31*) 1938; Morinaga and Aoki (*15*) 1938.
O. sativa × *officinalis*	24×24	Nandi (*31*) 1938, Ramanujam (*39*) 1937.
O. minuta × *officinalis*	48×24	Nandi (*31*) 1938–36.
O. minuta × *latifolia* (Cuba)	48×48	Morinaga and Aoki (*15*) 1938.

In the Annual Report of the Paddy Breeding Station, Coimbatore, India, for the year 1928–29, page 400, a statement indicates that a cross between *Oryza sativa* × *O. longistaminata* was obtained, and the nature of the F_1 is indicated. In a similar report for the year 1933–34, mention is made of F_3 plants of such a cross.

In all interspecific crosses reported to date with *Oryza sativa*, the F_1 plants were either completely sterile or were very low in fertility. The F_1 plants of the cross *O. latifolia* × *O. minuta* and reciprocal, both tetraploid species, according to Morinaga and Aoki (*15*), were also relatively low in fertility. Ramanujam (*39*) planted self-sterile F_1 plants of the cross *O. sativa* × *O. officinalis* (diploids) close to *O. sativa* plants, and the plants set 10 seeds, probably by cross-pollination of the F_1 with *O. sativa*. Six of the 10 seeds germinated and produced sterile plants, all of which proved to be triploids with the $2n$ chromosome number 36. This was assumed to be due to the formation of diploid gametes by double division of the univalent chromosomes in the F_1 plants.

DISCUSSION

The fact that sterility often occurs in intervarietal crosses in cultivated rice, even though the parents have the same number of chromosomes, and in the F_1 plants of all the interspecific crosses with *Oryza* thus far reported, indicates that the chromosomes of the cultivated varieties have probably undergone changes due to gene mutations and genic rearrangement to such an extent that they often are incompatible when crossed. A knowledge of some of the chromosomal aberrations, gene mutations, and apparent genic rearrangements, mentioned in this paper and in the literature cited, that have occurred in cultivated varieties in a relatively short period of time tends to confirm this point of view. It is possible that the cultivated rice varieties classed by Kato et al (*10*) as *O. sativa japonica* and *O. sativa indica* represent varieties of the same origin that have been grown under different environmental conditions so long that, owing to an accumulation of gene mutations and genic rearrangements within the chromosomes, they now are largely incompatible when crossed.

Haploid rice plants probably arise from the parthenogenetic development of an egg cell, as appears to be the case with most haploids in higher plants. Aneuploids have been shown to arise in rice by pollination of self-sterile triploid by diploid plants (*23*). The aneuploids reported in this paper also are believed to have originated in this way, because the maternal plants were very similar to plants that later proved to be triploids. Triploids are believed to originate by the union of haploid and diploid gametes; by the fusion of a diploid egg and a haploid male gamete, as in a cross of tetraploid × diploid plants; or by fertilization of a haploid egg by two male nuclei (dispermy). Diploid gametes have been reported in *Oryza sativus* by Nandi (*29, 30*), and by Ramanujam (*39*); and also in *O. officinalis* by Nandi (*31*). Tetraploid plants may arise by the fusion of diploid gametes or by somatic chromosome doubling. Ramanujam (*39*), in a cross of *O. sativa* × *officinalis*, obtained a sterile F_1 hybrid; from the F_1 backcrossed to *O. sativa*, six triploid plants were obtained. Cytological studies of the triploids indicated the presence of two sets of *O. sativa* chromosomes and one set of *O. officinalis*, which according to Ramanu-

jam (*39, p. 252*) "is the first case in plants of experimental demonstration of the origin of polyploidy through formation of diploid gametes by double division of the chromosomes."

This chromosome study of the rice varieties grown in the United States has shown that there are present in them nearly all the aberrant forms that have been described in rice in India and Japan. However, it offers neither confirmation nor denial of the view put forth by Kuwada (*11*) and Sakai (*51*) and supported by Nandi (*28*) that the basic chromosome number for rice is 5. Ramiah, Parthasarathi, and Ramanujam (*47*) found some pollen mother cells in a haploid plant with six bivalent chromosomes at diakinesis, a discovery that does not exclude the possibility that 6 may be the basic number. The X-ray studies of Ramiah, Parthasarathi, and Ramanujam (*48*) have shown that rings and chains of four chromosomes result from induced transmutations. If such groupings are readily produced by induced changes, why, as Morinaga and Fukushima (*19*) ask, do the chromosomes of the haploids fail to show more bivalents and larger chromosome groups in the meiotic divisions if the basic number for rice is 5?

The early prophase threads of rice chromosomes do not seem very promising for a morphological study of the individual chromosomes. It is unfortunate, however, that lack of time and the difficulty of obtaining good material of sterile diploids and of trisomics prevented a more careful search in these groups for such morphological features as might explain the aberrant behavior of the pollen mother cells in pollen-sterile plants. A careful examination of chromosomes might disclose characteristics that would be useful in determining which chromosomes were present in triplicate in the trisomic plants.

SUMMARY

Haploids in rice found at several experiment stations, and haploid, sterile diploid, aneuploid, triploid, and tetraploid plants in the Caloro variety of rice that arose as mutations at the Biggs Rice Field Station, Biggs, Calif., are described briefly. The polyploid types appear to be of no practical value.

Cytological studies of these mutants show the presence in the United States of nearly all the aberrant forms reported in rice in Japan and India.

Comparative studies of the gametic tissue of normal and sterile diploid plants have shown that the sterile plants have an abnormal meiotic behavior despite their diploid chromosome number.

LITERATURE CITED

(1) Gotoh, Kazuo, and Okura, Eiji.
1933. A preliminary note on cytological studies of oryza. Soc. Trop. Agr. Jour. 5: 363–364, illus.

(2) ——— and Okura, E.
1935. Cytological and genetical studies of oryza. Jap. Jour. Genet. 11: 130–131.

(3) Hedayetullah, S.
[1934.] A cytogenetical study of the behaviour of nucleoli in the pollen mother cells of three varieties of rice (oryza sativa l.) in the parent plants and their hybrids. Imp. Bur. Plant Genet. (Crops other than Herbage), Plant Breed. Abs. 4: 11.

(4) HEYN, A. N. J.
1936. CYTOLOGISCHE ONDERZOEKINGEN AAN ENKELE TROPISCHE CULTUURGEWASSEN EN DE BETEEKENIS VAN DERGELIJKE ONDERZOEKINGEN VOOR DE PLANTENVEREDELING. Landbouw 12: 11–42, illus. [In Dutch. English summary, pp. 41–42.]
(5) ———
1938. DU NOMBRE DE CHROMOSOMES CHEZ QUELQUES VÉGÉTAUX EN CULTURE AUX INDES NÉERLANDAISES (COFFEA, CEIBA, ORYZA, DERRIS, ET PALAQUIUM). Buitenzorg Jard. Bot. Ann. 48: 103–120, illus.
(6) HIRAYOSHI, ISAO.
1937. [SPECIES HYBRIDS BETWEEN CULTIVATED RICE AND WILD RICE (ORYZA LATIFOLIA) WITH SPECIAL REFERENCE TO THEIR STERILITY AND MATURATION DIVISION.] (Preliminary Note) Jap. Jour. Genet. 13: 59–60. [In Japanese.]
(7) ———
1937. CHROMOSOME-NUMBER IN ORYZOIDEAE. (Preliminary Note) Jap. Jour. Genet. 13: 215–216. [In Japanese. English summary, p. 216.]
(8) HOROVITZ, S., and POGLIAGA, H.
1934. NÚMERO DE CROMOSOMAS DE ORYZA SUBULATA NEES. Rev. Argentina de Agron. 1: 230–231, illus.
(9) ICHIJIMA, KICHITARO.
1934. ON THE ARTIFICIALLY INDUCED MUTATIONS AND POLYPLOID PLANTS OF RICE OCCURRING IN SUBSEQUENT GENERATIONS. Imp. Acad. Japan, Proc. 10: 388–391, illus.
(10) KATO, SHIGEMOTO, KOSAKA, HIROSI, HARA, SHIROKU, MARUYAMA, YASHIO, and TAKIGUCHI, YOSHISUKE.
1930. ON THE AFFINITY OF THE CULTIVATED VARIETIES OF RICE PLANTS, ORYZA SATIVA L. Kyushu Imp. Univ., Dept. Agr. Jour. 2: [241]–276.
(11) KUWADA, YOSHINARI.
1910. A CYTOLOGICAL STUDY OF ORYZA SATIVA L. Bot. Mag. [Tokyo] 24: [267]–281, illus.
(12) MATUSIMA, K.
1935. CHROMOSOME DOUBLING IN THE RICE PLANTS TREATED WITH HIGH TEMPERATURE. Jap. Jour. Genet. 11: 235–237, illus. [In Japanese.]
(13) MIYAZAWA, BUNGO.
1935. STUDIES ON MUTATIONS IN RICE. Miyazaki Col. Agr. and Forestry Bul. 7: 1–110, illus. [In Japanese. English summary, pp. 101–109.]
(14) MORINAGA, T[oshitaro].
1934. SOME OBSERVATIONS ON ORYZA MINUTA PRESL. Jap. Jour. Genet. 10: [91]–92, illus. [In Japanese.]
(15) ——— and AOKI, M.
1938. [SPECIES HYBRIDS OF RICE.] Agr. and Hort. [Tokyo] 13: 1993–2005, illus. [In Japanese. Translation by Saburo Katsura in U. S. Dept. Agr. Libr.]
(16) ——— and FUKUSHIMA, E.
1931. CHROMOSOME NUMBERS OF CULTIVATED PLANTS III. Bot. Mag. [Tokyo] 45: 140–145, illus.
(17) ——— and FUKUSHIMA, EIJI.
1931. PRELIMINARY REPORT ON THE HAPLOID PLANT OF RICE, ORYZA SATIVA L. Imp. Acad. Japan, Proc. 7: 383–384, illus.
(18) ——— and FUKUSHIMA, EIJI.
1932. SOME OBSERVATIONS ON THE MICROSPOROGENESIS OF THE HAPLOID PLANT OF RICE. Imp. Acad. Japan, Proc. 8: 403–405, illus.
(19) ——— and FUKUSHIMA, EIJI.
1934. CYTO-GENETICAL STUDIES ON ORYZA SATIVA L. I. STUDIES ON THE HAPLOID PLANT OF ORYZA SATIVA. Jap. Jour. Bot. 7: [73]–106, illus.
(20) ——— and FUKUSHIMA, EIJI.
1935. CYTO-GENETICAL STUDIES ON ORYZA SATIVA L. II. SPONTANEOUS AUTOTRIPLOID MUTANTS IN ORYZA SATIVA L. Jap. Jour. Bot. 7: [207]–225, illus.

319107—41——2

(21) MORINAGA, T[oshitaro] and FUKUSHIMA, E.
1936. [OBSERVATIONS ON THE AUTOTETRAPLOID RICE PLANTS.] (Preliminary Note) Jap. Jour. Genet. 12: 59. [In Japanese.]
(22) ——— and FUKUSHIMA, E.
1937. CYTO-GENETICAL STUDIES ON ORYZA SATIVA L. III. SPONTANEOUS AUTOTETRAPLOID MUTANTS IN ORYZA SATIVA L. Jap. Jour. Bot. 9: 71–94, illus.
(23) NAKAMORI, EIITI.
1932. ON THE APPEARANCE OF THE TRIPLOID PLANT OF RICE, ORYZA SATIVA L. Imp. Acad. Japan, Proc. 8: 528–529, illus.
(24) ———
1933. ON THE OCCURRENCE OF THE TETRAPLOID PLANT OF RICE, ORYZA SATIVA L. Imp. Acad. Japan, Proc. 9: 340–341, illus.
(25) NAKAMURA, SEISUKE.
1931. CYTOLOGICAL OBSERVATIONS IN TWO FORMS OF STERILE RICE PLANT. Crop Sci. Soc. Japan, Proc. 3: 259–265. [In Japanese.]
(26) ———
1933. [HAPLOID PLANTS IN RICE.] Jap. Jour. Genet. 8: [223]–227, illus. [In Japanese.]
(27) [NAKATOMI, SADAO.]
1923. [ON THE DIFFERENCES OF CHROMOSOMES IN VARIOUS RACES AND MUTANTS OF THE RICE PLANT.] Jap. Jour. Genet. 2: [107]–115. illus. [In Japanese. English summary in Jap. Jour. Bot. 2: (19).]
(28) NANDI, H. K.
1936. THE CHROMOSOME MORPHOLOGY, SECONDARY ASSOCIATION AND ORIGIN OF CULTIVATED RICE. Jour. Genet. 33: [315]–336, illus.
(29) ———
1937. CYTOLOGICAL INVESTIGATIONS OF RICE VARIETIES. Cytologia 8: 277–305, illus.
(30) ———
1937. EFFECT OF TEMPERATURE ON THE FORMATION OF DIPLOID GAMETES IN ORYZA SATIVA. 24th Indian Sci. Cong., Hyderabad, Proc. 1937: 268.
(31) ———
1938. INTER-SPECIFIC HYBRIDISATION IN ORYZA. I. CYTOGENETICAL EVIDENCE OF THE HYBRID ORIGIN OF ORYZA MINUTA PRESL. Bose Res. Inst., Calcutta, Trans. (1935–36) 11: 99–121, illus.
(32) OKURA, EIJI.
1937. CYTO-GENETICAL STUDIES ON ORYZA. A PRELIMINARY NOTE ON INTER-SPECIFIC HYBRID BETWEEN O. SATIVA L. AND O. MINUTA PRESL. Jap. Jour. Genet. 13: 212–214, illus. [In Japanese.]
(33) PARTHASARATHY, N.
1938. CYTOGENETICAL STUDIES IN ORYZEAE AND PHALARIDEAE. I. CYTOGENETICS OF SOME X-RAY DERIVATIVES IN RICE (ORYZA SATIVA L.). Jour. Genet. 37: 1–40, illus.
(34) ———
1938. CYTOLOGICAL STUDIES IN ORYZEAE AND PHALARIDEAE. II. FURTHER STUDIES IN ORYZA. Cytologia 9: 307–318, illus.
(35) ———
1939. CYTOGENETICAL STUDIES IN ORYZEAE AND PHALARIDEAE. III. CYTOLOGICAL STUDIES IN PHALARIDEAE. Ann. Bot. [London] (n.s.) 3: 43–76, illus.
(36) RAMANUJAM, S.
1935. MALE STERILITY IN RICE. (Research Notes) Madras Agr. Jour. 23: 190.
(37) ———
1936. CHROMOSOME STUDIES IN THE ORYZEAE. Brit. Assoc. Adv. Sci. Blackpool, Ann. Rpt., 1936: 418–419.
(38) ———
1937. CYTOGENETICAL STUDIES IN THE ORYZEAE. II. CYTOLOGICAL BEHAVIOUR OF AN AUTOTRIPLOID IN RICE (ORYZA SATIVA). Jour. Genet. 35: [183]–221, illus.
(39) ———
1937. CYTOGENETICAL STUDIES IN THE ORYZEAE. III. CYTOGENETICAL BEHAVIOUR OF AN INTER-SPECIFIC HYBRID IN ORYZA. Jour. Genet. 35: [223]–258, illus.

(40) RAMANUJAM, S.
1938. CYTOGENETICAL STUDIES IN THE ORYZEAE. I. CHROMOSOME STUDIES IN THE ORYZEAE. Ann. Bot. [London] (n.s.) 2: 107–125, illus.
(41) ——— AND PARTHASARATHY, N.
1935. AN ASYNAPTIC MUTANT IN RICE (ORYZA SATIVA). Indian Acad. Sci. Proc., Sect. B, 2: 80–87, illus.
(42) RAMIAH, K.
1931. PRELIMINARY INVESTIGATIONS ON THE OCCURRENCE OF STERILITY IN RICE (O. SATIVA). Agr. and Livestock in India 1: 414–426, illus.
(43) ———
1934. RICE RESEARCH IN MADRAS. Current Sci. [India] 3: 34–36.
(44) ———
1936. RICE GENETICS. Assoc. Econ. Biol. Proc. (1935) 3: [51]–61.
(45) ——— PARTHASARATHI, N., and RAMANUJAM, S.
1933. HAPLOID PLANT IN RICE (ORYZA SATIVA). Current Sci. [India] 1: 277–278, illus.
(46) ——— PARTHASARATHI, N., and RAMANUJAM, S.
1933. A TRIPLOID PLANT IN RICE (ORYZA SATIVA). Current Sci. [India] 2: 170–171, illus.
(47) ——— PARTHASARATHI, N., and RAMANUJAM, S.
1934. A HAPLOID PLANT IN RICE. Indian Bot. Soc. Jour. 13: 153–164, illus.
(48) ——— PARTHASARATHI, N., and RAMANUJAM, S.
1935. CHROMOSOME RING IN X-RAYED RICE. Assoc. Econ. Biol. Proc. (1934) 2: [1]–4, illus.
(49) ——— PARTHASARATHY, N., and RAMANUJAM, S.
1935. A TETRAPLOID PLANT IN WILD RICE (ORYZA LONGISTAMINATA). Indian Acad. Sci. Proc., Sect. B, 1: 565–570, illus.
(50) RAU, N. S.
1929. FURTHER CONTRIBUTIONS TO THE CYTOLOGY OF SOME CROP PLANTS OF SOUTH INDIA. Indian Bot. Soc. Jour. 8: 201–206, illus.
(51) SAKAI, KAN-ICHI.
1935. CHROMOSOME STUDIES IN ORYZA SATIVA L. I. THE SECONDARY ASSOCIATION OF THE MEIOTIC CHROMOSOME. Jap. Jour. Genet. 11: [145]–156, illus. [In Japanese. English summary, p. 154.]
(52) SELIM, A. G.
1930. A CYTOLOGICAL STUDY OF ORYZA SATIVA L. Cytologia 2: 1–26, illus.
(53) SETHI, B. L.
1937. CYTOLOGICAL STUDIES IN PADDY VARIETIES. Indian Jour. Agr. Sci. 7: 687–706, illus.
(54) TAKAHASHI, HIROYUKI.
1936. ÜBER DIE AUS DEN HAPLOIDEN REISPFLANZEN HERVORGEGANGENEN INDIVIDUEN. Crop Sci. Soc. Japan, Proc. 8: 355–363, illus. [In Japanese. German summary in Jap. Jour. Bot. 9: (23), 1937.]

KNOB POSITIONS ON TEOSINTE CHROMOSOMES[1]

By A. E. LONGLEY

Associate botanist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

The meiotic prophases of corn (*Zea mays* L.) and its near relatives are unusually favorable for critical studies of the morphological characters of the chromosomes, since the chromosomes at the early thread stages of the pollen mother cells are sharply differentiated by iron-aceto-carmine stain. It is on these paired threads, before contraction sets in to obscure the finer markings of the pachytene chromosomes, that knobs are most prominent, and it is the presence of these and other distinctive characters visible on the earlypro phase chromosomes that has made this group of plants so useful for chromosome studies.

The writer (*7*)[2] has recently published on the relationship between knob position and knob frequency in corn chromosomes, showing that each chromosome arm has a most favorable knob-forming position and that the frequency of occurrence of a knob depends upon its proximity to this most favorable position. The available data suggested a similar relationship for the knobs on annual teosinte (*Euchlaena mexicana* Schrad.) chromosomes. Recently additional data on the morphological characters of teosinte chromosomes have been obtained and have been analyzed with respect to the frequency with which knobs are found at different loci on the chromosomes. These data substantiate the earlier suggested relationship between knob position and knob frequency.

These data also seem to show a division of all teosintes from Guatemala into two groups, a northern group centering around Huehuetenango, and a southern group centering around Progreso and Jutiapa.

Finally, these data indicate that the chromosomes of the northern group of teosinte are more like corn than those of the southern group.

MATERIAL AND METHODS

The material for this study of the morphological characters of the midprophase chromosomes of Guatemalan teosinte was taken from plants grown in the greenhouse at Washington, D. C., at Charleston, S. C., at Chapman Field and Canal Point, Fla., and at Torrey Pines, Calif.

The seeds for these plantings came from Nojoyá and San Antonio Huixta, in the Department of Huehuetenango, northern Guatemala; and from Moyuta, Progreso, Jutiapa, Retana, and Chilamates, in the Department of Jutiapa, southern Guatemala (*4, map*). The seeds from the Department of Huehuetenango will be referred to as the northern group, and the seeds from the Department of Jutiapa as the southern group. In the latter group is also included Florida teosinte, which

[1] Received for publication December 2, 1940.

[2] Italic numbers in parentheses refer to Literature Cited, p. 413.

historical evidence indicates originated near Progreso, in southern Guatemala.

The methods of preparing and studying pollen mother cells were the same as those outlined recently (*7*).

CHROMOSOME LENGTH

The length of any chromosome depends upon the stage at which it is measured. During the prophase of the first reduction division the chromosomes of teosinte change from the long threads of the early prophase to the compact bodies found at diakinesis. This change frequently reduces the length of a chromosome from 80μ–100μ to 8μ–10μ. Care was taken to eliminate as much variation in chromosome length as possible, but in spite of all precautions the measurements varied much. The means for each chromosome (table 1), although derived from many measurements, still show rather large errors.

It is only after months of study that each of the 10 corn chromosomes can be identified in the early thread stage. This problem is even more difficult with teosinte chromosomes, since knobs, the positions of which help materially in identifying corn chromosomes, are all terminal in teosinte. Identification, therefore, of the teosinte chromosomes depends upon their relative lengths, the relative length of the two arms of each, and upon other minor but stable characteristics that continued study has shown to distinguish certain chromosomes.

The homology between teosinte and corn chromosomes has been observed in a previous study (*5*) of F_1 teosinte-corn hybrids, in which it was found that the numbers assigned to corn chromosomes, based on total length, are with few exceptions equally applicable to teosinte chromosomes.

The extended threadlike chromosomes of both teosinte and corn are found to differ in length, in addition to the usual variation, because of the presence of knobs. The increase in length of a corn chromosome due to the presence of a knob has been previously suggested (*5*), but it has been tested only recently by actual measurements. From this test it was found that each of the 15 chromosome arms known to bear knobs is appreciably shorter when knobs are absent than when knobs are present (table 1). The writer suspects that some of this increase in length of knob-bearing arms may be mechanical. The elastic of a slingshot stretches when a stone is in it and in somewhat the same manner the longer chromosome arms may be stretched by the presence of a knob. Such a mechanical increase in chormosome length may account for the fact that in F_1 teosinte-corn hybrids (*5*) the terminal knobs of the teosinte homologue were frequently seen reaching beyond the end of the corn chromosome.

At the time the measurements given in table 1 were made it was not considered that differences in chromosome length might be due at least in part to the presence or absence of a knob. Since the teosinte material collected had relatively few arms without knobs, an estimate of what the length of the arms would be without knobs was obtained by subtracting from the mean length the mean knob size for the particular arm.

TABLE 1.—*Mean length and position of fiber attachment of midprophase chromosomes*

Chromosome No.	Measurements for —			Mean length for —			Mean length (without knobs) for —			Mean fiber-attachment position for [1] —			Mean fiber-attachment position (without knobs) for [2] —		
	Southern teosinte	Northern teosinte	Corn	Southern teosinte	Northern teosinte	Corn	Southern teosinte	Northern teosinte	Corn	Southern teosinte	Northern teosinte	Corn	Southern teosinte	Northern teosinte	Corn
	Number	*Number*	*Number*	μ	μ	μ	μ	μ	μ						
I	90	126	343	80.52±1.61	80.97±1.63	82.40±0.90	78.60	79.58	79.74	0.435	0.434	0.435	0.422	0.425	0.437
II	68	133	306	70.59±1.46	68.55±1.26	66.50± .75	66.82	65.21	63.43	.444	.433	.444	.437	.427	.451
III	93	120	354	62.36±1.20	63.13±1.20	62.00± .74	59.79	61.02	60.01	.358	.347	.331	.330	.326	.339
IV	55	115	342	61.11±1.64	60.31±1.29	58.78± .60	57.97	57.64	57.03	.391	.382	.382	.379	.374	.393
V	49	111	320	58.58±1.85	58.02±1.16	59.82± .68	54.47	55.41	58.57	.473	.462	.458	.469	.457	.467
VI	80	133	377	43.65± .94	45.64± .79	48.73± .51	41.58	44.07	46.61	.229	.241	.244	.241	.250	.256
VII	105	129	367	47.98± .87	48.79± .78	46.78± .51	45.46	46.63	44.42	.270	.299	.266	.285	.274	.280
VIII	112	114	361	47.84± .93	46.60± .78	47.48± .53	47.16	43.86	44.79	.246	.309	.237	.250	.284	.251
IX	113	135	416	42.55± .77	42.88± .75	43.24± .43	39.87	40.68	41.51	.294	.331	.352	.314	.297	.333
X	112	169	391	37.50± .70	37.33± .50	36.93± .36	35.03	37.06	36.93	.246	.265	.266	.263	.267	.266

[1] Length of short arm relative to entire chromosome including knobs.
[2] Length of short arm relative to entire chromosome excluding knobs.

All recent studies of knobs have included, when possible, an indication of the size of the individual knobs, according to 10 steps for classification, beginning with the smallest, having a diameter of 0.33μ, and ending with the largest, with a diameter of 3.3μ. From this classification the mean size of each knob and the mean size per plant of each knob were calculated and are given in table 2, columns 9 and 10, and 11 and 12, respectively. The mean size per plant is the number of microns that each knob increases the length of the arm on which it occurs. Thus, the length of each chromosome, excluding the knobs, was computed from the total length (table 1). Such a calculation disregards the mechanical elongation of a chromosome due to the presence of knobs, if such elongation has occurred.

TABLE 2.—*Mean size and frequency of chromosome knobs in Guatemalan teosinte*

Chromosome		Measurements for—				Knob frequency in—		Mean knob size for—		Mean knob size per plant at each position for—	
		Southern teosinte		Northern teosinte							
No.	Arm	With knob	Without knob	With knob	Without knob	Southern teosinte	Northern teosinte	Southern teosinte	Northern teosinte	Southern teosinte	Northern teosinte
		Number	*Number*	*Number*	*Number*	*Percent*	*Percent*	μ[1]	μ[1]	μ[1]	μ[1]
X	Short	0	53	0	68	0	0	0	0	0	0
VIII	do	0	47	55	1	0	98.21	0	1.97	0	1.93
VI	do										
VII	do	0	45	53	8	0	86.89	0	2.09	0	1.82
IX	do	0	53	61	1	0	98.39	0	2.17	0	2.14
III	do	41	0	55	0	100.00	100.00	2.57	2.03	2.57	2.03
IV	do	27	0	60	0	100.00	100.00	1.89	1.49	1.89	1.49
X	Long	53	0	35	33	100.00	51.47	2.47	.53	2.47	.27
V	Short	32	0	50	0	100.00	100.00	2.16	1.45	2.16	1.45
IX	Long	52	1	8	54	98.11	12.90	2.73	.48	2.68	.06
II	Short	33	0	62	0	100.00	100.00	2.14	1.85	2.14	1.85
V	Long	31	1	46	4	96.87	92.00	2.01	1.26	1.95	1.16
VII	do	45	0	18	43	100.00	29.51	2.52	1.15	2.52	.34
I	Short	43	0	59	0	100.00	100.00	1.92	1.37	1.92	1.37
VIII	Long	36	11	44	12	76.60	78.57	.89	1.03	.68	.81
IV	do	20	7	56	4	74.07	93.33	1.69	1.27	1.25	1.18
VI	do	38	0	64	0	100.00	100.00	2.07	1.57	2.07	1.57
II	do	32	1	59	3	96.97	95.16	1.66	1.57	1.61	1.49
III	do	0	41	7	49	0	12.50	0	.66	0	.08
I	do	0	43	1	58	0	1.69	0	.33	0	.01
Mean per knob								2.05	1.35	1.99	1.17
Mean per plant										25.91	21.05

[1] Approximate diameter of knob.

These efforts to find the length of each chromosome, exclusive of knobs, are not intended to suggest that knobless chromosomes represent the characteristic chromosomes of teosinte or corn. They were made in order to arrive at a basic length that would prove most useful in comparing chromosomes from the two teosinte groups with each other and with corn.

The mean chromosome lengths, both with and without knobs, are given in table 1 for all chromosomes of the two teosinte groups and of corn. These data show that the chromosome with the greatest length difference is VI. It had been found in an earlier study (*5*) of F_1 toesinte-corn hybrids that chromosomes VI from teosinte and corn

are equal in length or that chromosomes VI of teosinte may be even longer than chromosome VI of corn, but never shorter as the lengths in table 1 would indicate. Consequently, it would seem that the data presented in table 1 on chromosome lengths give no definite proof that there is a significant difference in the lengths of any of the 10 homologues as they appear in either of these two teosinte groups and in corn.

The relative length of the short arm of each chromosome to its whole length is given in table 1 under the heading "Mean fiber-attachment position." When measurements that included the length of the knobs were used in determining these fractions for the two teosinte groups and for corn, it was found that in some cases the three homologous chromosomes had quite different positions for the fiber attachment.

However, when these fractions were determined from measurements from which the length of the knobs had been deducted, the large differences in fiber-attachment positions for each homologue were reduced, and the whole array of fiber-attachment positions given in the last three columns of table 1 suggests, with the possible exceptions of chromosomes IX and VIII, no real differences between the chromosomes from either of the teosinte groups or from corn.

If the mean lengths of each chromosome arm of the two teosinte groups are compared (table 3), it will be seen that the mean lengths from measurements without knobs show only those for the long arm of chromosome VIII to be appreciably different. When this comparison includes corn as well, there are found to be differences in the long arms of chromosomes VIII, VI, V, and II and the short arm of V.

TABLE 3.—*Mean length of arms of midprophase chromosomes of teosinte and corn*

Chromosome		Length (with knobs) for—			Length (without knobs) for—		
No.	Arm	Southern teosinte	Northern teosinte	Corn	Southern teosinte	Northern teosinte	Corn
		μ	μ	μ	μ	μ	μ
X	Short	9.22±0.21	9.91±0.18	9.81±0.10	9.22	9.91	9.81
VIII	do	11.79± .28	14.38± .30	11.26± .15	11.79	12.45	11.26
VI	do	10.01± .26	11.00± .25	11.91± .15	10.01	11.00	11.91
VII	do	12.94± .25	14.60± .26	12.44± .16	12.94	12.78	12.42
IX	do	12.53± .23	14.21± .30	15.21± .18	12.53	12.07	13.82
III	do	22.32± .47	21.93± .46	20.51± .26	19.75	19.90	20.35
IV	do	23.88± .74	23.04± .53	22.47± .15	21.99	21.55	22.43
X	Long	28.28± .54	27.42± .37	27.12± .28	25.81	27.15	27.12
V	Short	27.68± .94	26.78± .59	27.37± .35	25.52	25.33	27.37
IX	Long	30.02± .59	28.67± .52	28.03± .30	27.34	28.61	27.69
II	Short	31.36± .72	29.71± .59	29.51± .35	29.22	27.86	28.60
V	Long	30.90± .95	31.24± .63	32.45± .38	28.95	30.08	31.20
VII	do	35.04± .68	34.19± .60	34.34± .39	32.52	33.85	32.00
I	Short	35.05± .75	35.18± .74	35.87± .39	33.13	33.81	34.88
VIII	Long	36.05± .71	32.22± .60	36.22± .42	35.37	31.41	33.53
IV	do	37.23±1.03	37.27± .83	36.31± .41	35.98	36.09	34.60
VI	do	33.64± .78	34.64± .63	36.82± .39	31.57	33.07	34.70
II	do	39.21± .83	38.84± .72	36.97± .45	37.60	37.35	34.83
III	do	40.04± .79	41.20± .82	41.49± .52	40.04	41.12	39.66
I	do	45.47± .95	45.78±1.26	46.52± .55	45.47	45.77	44.86

A summary of the data on length suggests a possible difference in lengths of chromosomes VIII, VI, V, and II between both groups of Guatemalan teosinte and corn. However, when the teosinte and corn chromosomes were within the same cell of an F_1 teosinte-corn hybrid, there was no difference in length between chromosome VI of corn and its homologue in teosinte.

CHROMOSOME KNOBS

It was the determination of knob frequency and position that revealed morphological differences in the chromosomes of corn from different geographical regions. Measurements have failed to show real differences in length between homologous chromosomes of teosinte from northern and from southern Guatemala. Consequently, a study has been made of the number, frequency, size, and position of the knobs on the chromosomes of these two teosinte groups.

All knobs on Guatemalan teosinte chromosomes are terminal. The few suggestions of internal knobs are never more prominent than large chromomeres and consequently are disregarded in this discussion.

Since the short arm of chromosome VI has no true knob in either teosinte or corn, but only a dark-staining body that is always present and is associated with the nucleolus organizer, this arm has been omitted in much of this consideration of chromosome knobs. Thus there are left 19 arms for examination. In northern Guatemalan teosinte, 18 of these arms have been seen with a terminal knob, while in teosinte from southern Guatemala only 13 have been found terminated by a knob.

The frequency of occurrence of each of the 13 knobs on the chromosomes of the southern group was found to be relatively high, and only 2 knobs have frequencies below 90 percent. In the northern group the frequency is more variable, although 13 of the 18 knobs have frequencies above 75 percent, the remaining 5 knobs occurring much less frequently. Table 2 shows the frequency of occurrence of each knob in each of the teosinte groups.

A comparative study of the first few plants from the two teosinte groups suggested that the knobs on the chromosomes of the southern group were much more prominent than those on the chromosomes of the northern group. To verify this suggested difference in knob size, the measurements of table 2 were made. These measurements are somewhat indefinite, since knobs are not spherical, nor even constant in shape; consequently it was the amount of knob material present and not in all cases the actual diameter or length of the longest axis that placed a knob in its size class.

According to this classification, the mean knob size for the northern group is 1.35 μ, while that of the southern group is 2.05 μ in diameter. Table 2 gives the mean size for each of the 18 knobs of the northern group and the 13 knobs of the southern group.

The data of table 2 revealed a relationship between knob frequency and knob size that was unexpected though in keeping with the writer's belief that the knob-forming positions that collect knob material most frequently will also collect a larger quantity of knob material. In the southern teosinte group, six of the eight knobs having a frequency of 100 percent are above the mean size, and of the five remaining knobs having frequencies below 100 percent, only one has a knob size above the mean. In the northern teosinte group the condition is similar, since all of the six knobs with a frequency of 100 percent are above the mean knob size, five of the six knobs with frequencies between 85 and 100 percent are above the mean knob size, and the six knobs with low frequencies are all below the mean size.

Since there seems to be an unquestionable relationship between knob frequency and knob size, it was thought that the two might be

combined by summing the measurements for each knob and dividing the total by the number of plants, both with and without knobs. This gave the mean amount of knob material going to each knob-forming position, and from these values the mean amount of knob material present in each plant was calculated. Table 2 gives these means for each knob position of the northern and of the southern teosintes and the mean amount of knob material per plant for each group.

From the data of table 2 it is apparent that in spite of the larger number of positions collecting knob material in teosinte from northern Guatemala, the group is characterized by a lower amount of knob material per plant and by a lower mean knob size than the teosinte from southern Guatemala.

If the 20 arms of the 10 chromosomes of teosinte from southern Guatemala are examined for the supposed relationship between knob position and knob frequency, it will be noticed that the 2 longest arms and the 4 shortest arms have no knobs; also that there is a slight reduction in knob frequency for the knobs terminating the third, fourth, and fifth longest arms. This suggests that the regions terminating the medium-length chromosome arms of teosinte from southern Guatemala are more favorably located for forming knobs than the regions terminating long and short arms.

Knob frequency in this group of teosinte seems to be determined by the length of the chromosome arms. In the pollen mother cells of this group, as in corn, the shorter chromosome arms seem too short and the longer too long to bear terminal knobs.

The relationship between knob position and knob frequency in corn (7) was shown by a curve that cut each arm at the region considered to be most favorable for knob formation. It seems unnecessary to use such a complicated curve to mark the position most favorable for knob formation in teosinte of southern Guatemala when the data on knob frequency are only sufficient to indicate that the most favorable position is beyond the end of the shorter chromosomes and is internal for the longer chromosomes.

The curve (straight line) that cuts each arm at its most favorable knob position and seems to answer the requirements of the data available is one that cuts the terminal point of an arm 25μ long. The most favorable knob positions on arms longer and shorter than 25μ were found by determining the difference between 25μ and the length of each arm. Then one-third of this difference was added to each arm if the arm was shorter or subtracted if the arm was longer than 25μ. By this empirical method the distance from the fiber attachment of the point most favorable for knob formation was found on each arm or arms produced.

Such a curve gives an increasing ratio between the distance from the fiber attachment and the most favorable knob-forming position and between 25μ and the distance from the most favorable knob position to the end of the chromosome when the chromosomes are longer and shorter than 25μ. It also gives comparable ratios to chromosomes too short or too long to bear terminal knobs.

Figure 1 shows the 20 chromosome arms of the southern teosinte group arranged in order of their length. On this figure are drawn two curves: (1) The dotted line at a position approximately that

used to mark the most favorable position for knob formation on the arms of corn chromosomes; and (2) the unbroken line, which is the curve thought to mark the most favorable knob position on each teosinte arm. A study of the points marked on each arm by these two curves will show that the one used in corn places the terminal region of most of the shorter arms and two of the longer arms of the southern teosinte group in a more favorable position for knob formation than the terminal region of the medium-length arms. On the other hand, the curve considered typical for teosinte (fig. 1) changes the relationship between the knobs and the most favorable knob-forming points so that the terminal regions of the medium-length chromosome arms

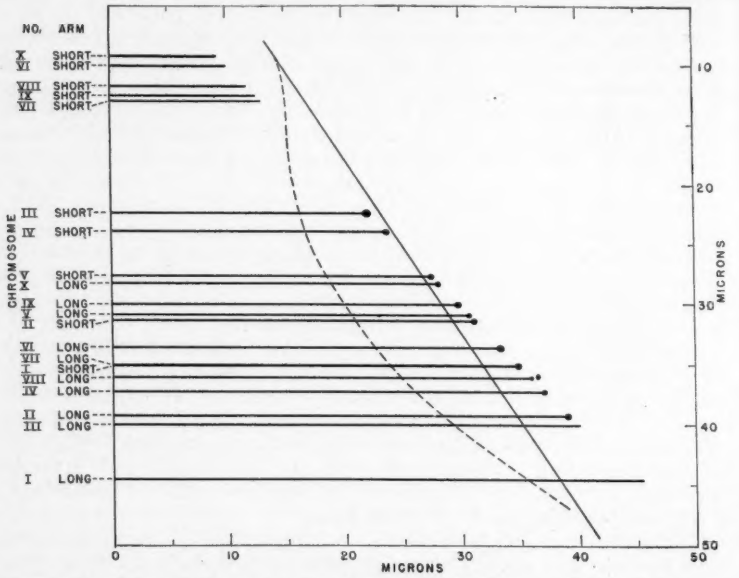

FIGURE 1.—Diagram of the 20 chromosome arms of southern Guatemalan varieties of teosinte, showing the curve that cuts each arm at the most favorable knob-forming position and the curve (broken) that cuts each arm at the most favorable knob-forming position for arms of corn chromosomes of these lengths.

are more favorably located for knob formation than are the terminal regions on the long and short arms.

Since the data discussed above indicate that the most favorable position for knob formation is farther removed from the fiber attachment in teosinte than in corn, it is thought the gradient along the chromosome arms of the southern teosinte group is different from that controlling knob formation on corn chromosomes. This change has resulted in reducing the possibility of finding knobs terminating the short arms of this group of teosintes and has put the terminal point of all medium-length arms in a much more favorable knob-forming position.

Turning again to table 2 and comparing the mean knob sizes for the two groups of teosinte, especially the values given when size and frequency have been combined, it will be noticed that some of the

larger knobs found in northern teosinte chromosomes are on arms that were without knobs in the southern teosinte group, while in other instances some of the smaller knobs are on arms that were terminated by large knobs in the southern group.

Selecting from table 3 all chromosome arms of the northern group with lengths between approximately 25μ and 35μ, it will be found that five of these have mean knob sizes near or above the average mean size and that four are appreciably below. This difference in knob size on arms of nearly the same length seems to assume significance when it is realized that the former belong to the longer chromosomes while the latter belong to the shorter chromosomes. This separation of the four shorter chromosomes from the longer chromosomes led

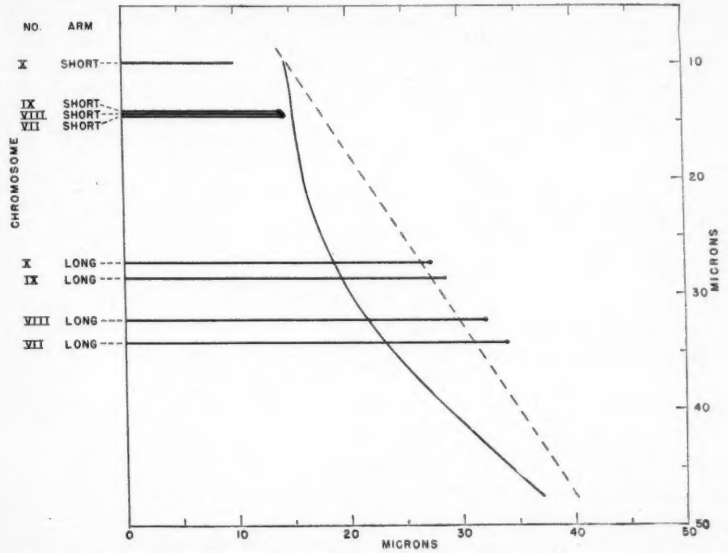

FIGURE 2.—Diagram of the eight chromosome arms of the four shortest chromosomes of northern Guatemalan teosinte, showing the curve that cuts each arm at the most favorable knob-forming position and the curve (broken) that cuts each arm at the most favorable knob-forming position for arms of southern Guatemalan teosinte chromosomes of these lengths.

to an inspection of the knobs on the short arms of these chromosomes. In the southern teosinte group these four arms were without knobs, but in the northern group, three of the four arms, instead of being without knobs, are terminated by large knobs.

Table 3 shows that these four arms in the northern group are not very different in length from the corresponding arms in the southern group. It would seem then that a different factor controls knob formation on the four shorter chromosomes of the northern teosinte group from that which controls knob formation on the chromosomes of the southern group.

By plotting the eight arms of the four shortest chromosomes of the northern Guatemalan teosinte (fig. 2) and superimposing the two curves

of figure 1, it is apparent that the terminal points of each arm, if controlled by the corn gradient, will not have the same ability to form knobs, and that it will be the shortest and the longest arms that will be without knobs or will have only small knobs. If the southern teosinte gradient were controlling the knob formation, it would be the longer arms that would be terminated by the largest knobs. The data on size of knobs clearly point to the presence of a gradient similar to that found in corn for the four shortest chromosomes of the northern teosinte group.

By plotting the 12 arms of the 6 longest chromosomes of the northern Guatemalan group (fig. 3) and superimposing two curves of figure 1, it is apparent that a gradient such as exists in the southern

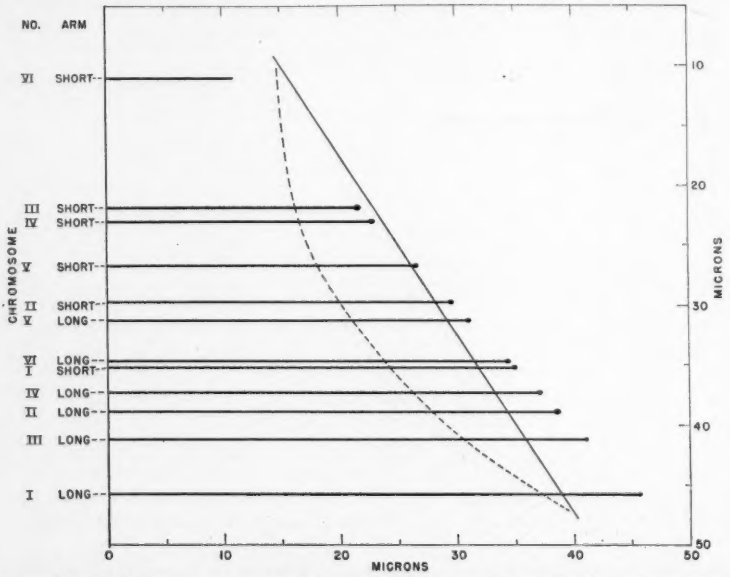

FIGURE 3.—Diagram of the 12 chromosome arms of the 6 longest chromosomes of northern Guatemalan teosinte, showing the curve that cuts each arm at the most favorable knob-forming position and the curve (broken) that cuts each arm at the most favorable knob-forming position for arms of corn chromosomes of these lengths.

teosinte group puts the terminal point of all but the longer arms in a favorable knob-forming position, while a gradient such as exists in corn puts the terminal points of the medium-length arms in the most unfavorable knob-forming position. The size and frequencies of the knobs on the arms of the 6 longest chromosomes of the northern group clearly indicate that knob formation is controlled by a gradient similar to that characteristic of the southern teosinte group.

If some of the northern teosinte chromosomes are controlled by one gradient and others by another, this would account for the fact that more knob-forming points are favorably located to collect knob material in this group than in the southern group. Therefore it is not alone the amount of the knob material available that determines

the number of knobs present but also the number of favorably located knob-forming points combined with the amount of knob material.

It is this comparison of the number, size, and position of the knobs of the two teosintes with each other and with corn that has led to the conclusion that the chromosomes of northern teosinte are more like the chromosomes of corn than are those of southern teosinte.

DISCUSSION AND CONCLUSIONS

The result of this comparison of the chromosomes of teosinte from southern Guatemala with those of northern Guatemala has led to the conclusion that the difference in length is so slight and the position of the fiber attachment is so nearly the same that there is no reason to believe that the homologues of the two groups are significantly different in these morphological characters.

The position and number of knobs of the northern group have been shown to be sufficiently different, however, from those of the southern group to distinguish readily plants of either group by the appearance of their chromosomes. This difference is most noticeable in the 4 shortest chromosomes and is thought to be due to a change in the factor or factors controlling chromosome gradient. Such a change is thought to give the 4 shorter chromosomes of the northern teosinte a gradient quite different from that of its 6 longest chromosomes and from that of all the chromosomes of the southern teosinte. The effect of the two gradients on the 10 chromosomes of the northern teosinte has been to put 18 knob-forming points into positions favorable for collecting knob material. Consequently, northern teosinte chromosomes have more knobs than southern teosinte, in spite of the fact that there is less knob material available in the pollen mother cells of the former than there is in the latter group.

Another possible difference in the homologous chromosomes of the two teosinte groups is in the linear arrangement of the genes. The writer has found in some recent unfinished studies that from one to four chromosome bridges are not unusual in pollen mother cells of the first division anaphases of F_1 Florida teosinte-corn hybrids, while O'Mara (*9*) finds constant and regular pairing between chromosomes of corn and those of teosinte from Nojoyá.

As in the comparison of the chromosomes of the two teosinte groups, so in the comparison between teosinte and corn chromosomes it is the position of the knobs that causes the greatest visible difference. The knobs on corn chromosomes are found much nearer the fiber attachment than the knobs on teosinte from southern Guatemala, and only in the four short chromosomes of teosinte from northern Guatemala does the position of the knobs suggest that their formation is controlled by the same factor or factors as in corn. These four chromosomes are the only ones with a gradient comparable to that of corn chromosomes.

Thus it may be concluded from this comparative study of the number and position of chromosome knobs that it is a difference in chromosome gradient which has made some of the chromosomes of the northern teosinte group appear more like their homologues of corn than like their homologues in the southern group.

The conclusions from this comparison of chromosome length of the two teosinte groups give no indication of a real difference in length of

the homologues in teosinte. Even the difference between teosinte and corn, previously suggested by the writer (*5*), may be due entirely to a physical lengthening of a chromosome by the presence of a large knob at a considerable distance from the fiber attachment.

It is thought, therefore, that, excluding the few inversions observed in crosses between teosinte from southern Guatemala and corn, a change of even a single-gradient controlling factor might suffice to change all the chromosomes of a teosinte from the southern group to approximately those of the northern group or even to those of corn or vice versa.

The similarity and difference between the chromosomes of teosinte and corn have been the subject of intensive study by several investigators (*1*, *2*, *3*). Such studies have been carried out from the point of view of the geneticist, the cytologist, and the cytogeneticist.

The outcome of these studies has been well summarized in the mind of the writer by the following statement of Arnason (*1*): "The results suggest that gene changes rather than changes in the gross structure of the chromosomes may have been chiefly responsible for the difference of maize and annual teosinte."

If this comparison between teosinte chromosomes from different geographical regions and between the chromosomes of teosinte and corn be extended to include the annual teosintes of Mexico, it will be found that the chromosomes of the Mexican teosintes are more cornlike than the chromosomes of teosintes from Guatemala. Some of the investigators of the chromosomes of Mexican teosintes have failed, however, to recognize that according to competent judges these teosintes are admixtures of teosinte and corn. The writer also would like to consider that Mexican teosintes have not been contaminated with corn and thus have available for comparison a graded series from the least cornlike types of southern Guatemala to the most cornlike types from Mexico. Unquestionably, such a series of teosintes no longer exists in isolated regions that have kept the teosintes free from corn contamination, but it does not seem unreasonable to the writer that, with a common ancestral type in which a few major mutations have occurred, such a graded series in teosinte might have existed at some earlier period.

Euchlaena perennis Hitchc., a perennial teosinte that has been almost overlooked in a recent publication (*8*) on the origin of corn and its relatives, seems to deserve serious consideration in any discussion of the relationship between teosinte and corn. Unfortunately, this species is known only in its tetraploid form. Even in this form it seems to show that true teosintes may be no further removed from corn than they are from each other. A comparison of the prophases of the F_1 of *E. perennis* × corn with those of the F_1 of *E. perennis* × southern Guatemalan teosinte shows that there is a partial synapsis between the teosinte and corn chromosomes in the former hybrid, while in the latter there is little or no synapsis between the chromosomes of the two teosintes.

The position and number of chromosome knobs have proved useful in distinguishing the two groups of teosintes from Guatemala. The almost complete absence of knobs on perennial teosinte (*5*) clearly places this teosinte in a class by itself.

The amount of knob material in the pollen mother cells in the teosintes from three isolated localities shows that knob material is most

abundant from teosintes of the southern region and is reduced to practically zero in perennial teosinte from central Mexico. A change in geographical location of a few hundred miles has affected the knob material in teosintes in much the same manner as a change from Mexico and Central America to localities both north (*6*) and south has reduced the amount of knob material in the pollen mother cells of corn.

SUMMARY

In gross morphological characters, such as chromosome length and position of fiber attachments, the chromosomes of teosinte are very similar to each other and to their homologues in corn.

The position and number of the chromosome knobs of teosinte have characteristic differences that make teosintes from different localities reasonably distinguishable.

These distinguishing features are of the type that indicates that teosintes differ in their relationship to each other and to corn, the teosintes of northern Guatemala being slightly more cornlike than those from southern Guatemala, while perennial teosinte from Mexico is thought to have chromosomes even more similar to those of corn.

Considering only the morphological characters of the chromosomes of teosinte and corn, it seems possible that a few gene mutations in the original type of teosinte may have given rise to a form that could have become the progenitor of corn.

LITERATURE CITED

(1) Arnason, T. J.
1936. cytogenetics of hybrids between zea mays and euchlaena mexicana. Genetics 21: 40–60, illus.

(2) Beadle, G. W.
1932. studies of euchlaena and its hybrids with zea. I. chromosome behavior in euchlaena mexicana and its hybrids with zea mays. Ztschr. f. Induktive Abstam. u. Vererbungslehre 62: [291]–304, illus.

(3) Emerson, R. A., and Beadle, G. W.
1932. studies of euchlaena and its hybrids with zea. II. crossing over between the chromosomes of euchlaena and those of zea. Ztschr. f. Induktive Abstam. u. Vererbungslehre 62: [305]–315.

(4) Kempton, J. H., and Popenoe, Wilson.
1937. teosinte in guatamala. report of an expedition to guatemala, el salvador, and chiapas, mexico. Carnegie Inst. Wash., Pub. No. 483: 199–217, illus.

(5) Longley, A. E.
1937. morphological characters of teosinte chromosomes. Jour. Agr. Res. 54: 835–862, illus.

(6) ———
1938. chromosomes of maize from north american indians. Jour. Agr. Res. 56: 177–195, illus.

(7) ———
1939. knob positions on corn chromosomes. Jour. Agr. Res. 59: 475–490, illus.

(8) Mangelsdorf, P. C., and Reeves, R. G.
1939. the origin of indian corn and its relatives. Texas Agr. Expt. Sta. Bul. 574, 315 pp., illus.

(9) O'Mara, Joseph G.
1938. cytological observations on zea-euchlaena hybrids. (Abstract) Genet. Soc. Amer. Rec. 7: 82–83.

319107—41——3

STUDIES ON THE PREPARATION OF MUSHROOM COMPOST [1]

By EDMUND B. LAMBERT

Pathologist, Division of Mycology and Disease Survey, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

In a previous paper [2] it was pointed out that there is a well-defined tendency for mushroom compost heaps to become stratified so that manure in various parts of a heap is composting under different conditions of temperature, moisture, and aeration. Undoubtedly these differences lead to diversities in the rate of decomposition, type of microbial flora, and chemical changes in separate areas of the heap.[3] [4] [5] [6] This more or less unavoidable heterogeneity in composting conditions causes a variability in the finished compost that makes experimental yield tests with mushrooms difficult and is an important source of variability in commercial yields.

The stratification of mushroom compost heaps into patterns with regard to physical conditions is dependent largely on the size, shape, and compactness of the heap. These patterns also change somewhat with the progress of the fermentation. The same principles, however, are basic to all heaps and may perhaps be best illustrated by a detailed description of the physical conditions in an average compost heap.

The pattern most commonly encountered is illustrated in figure 1. Usually four areas can be distinguished, having fairly distinct sets of physical conditions. The first (area *A*) is an outside layer from 2 to 6 inches thick, which varies in temperature from that of the surrounding air to 110° F. Compost in this area has a tendency to become dry on the sides of the heap, owing to excessive aeration; and in cool or wet weather it becomes wet on the top, owing to condensation or precipitation. The second layer (area *B*) lies just inside the first. It is well aerated, is moderately moist, and varies in temperature from 110° to 140°. The third (area *C*) extends around the inside of the heap like a huge doughnut from 2 to 4 feet from the sides and 1 to 3 feet from the top of the heap. This area contains the warmest compost in the heap, ranging from 150° to 180°. It is probable that the high temperatures are attained in this area because the compost in it is well insulated and receives sufficient air for oxidation without an excess to cause loss of heat by convection. The fourth distinct

[1] Received for publication December 31, 1940.

[2] LAMBERT, EDMUND B., and DAVIS, A. C. DISTRIBUTION OF OXYGEN AND CARBON DIOXIDE IN MUSHROOM COMPOST HEAPS AS AFFECTING MICROBIAL THERMOGENESIS, ACIDITY, AND MOISTURE THEREIN. Jour. Agr. Res. 48: 587–601 illus. 1934.

[3] GUHA SIRCAR, S. S., DE, S. C., and BHOWMICK, H. D. MICRO-BIOLOGICAL DECOMPOSITION OF PLANT MATERIALS. I. CHANGES IN THE CONSTITUENTS OF RICE STRAW (KANAKTARA) PRODUCED BY MICRO-ORGANISMS PRESENT IN SOIL SUSPENSION UNDER AEROBIC, ANAEROBIC AND WATERLOGGED CONDITIONS. Indian Jour. Agr. Sci. 10: 119–151, illus. 1940.

[4] WAKSMAN, SELMAN A., CORDON, T. C., and HULPOI, N. INFLUENCE OF TEMPERATURE UPON THE MICROBIOLOGICAL POPULATION AND DECOMPOSITION PROCESSES IN COMPOSTS OF STABLE MANURE. Soil Sci. 47: 83–113, illus. 1939.

[5] ——— UMBREIT, W. W., and CORDON, T. C. THERMOPHILIC ACTINOMYCETES AND FUNGI IN SOILS AND IN COMPOSTS. Soil Sci. 47: 37–61. illus. 1939.

[6] JENKINS, S. H. ORGANIC MANURES. Imp. Bur. Soil Sci. Tech. Commun. 33, 64 pp., illus. 1935.

region (area *D*) occupies the entire lower central part of the heap. The oxygen in this area is used up within 7 or 8 hours after the heap is "turned," so that the material is usually decomposing under anaerobic conditions.

To obtain a comparatively uniform compost, mushroom growers mix the manure from different parts of the heap at weekly intervals.

In spite of this mixing, however, there are noticeable dissimilarities in the appearance of the compost in the different areas, as indicated in figure 1, which persist during most of the composting process. For example, during the first and second turnings the manure in the anaerobic region (area *D*) is noticeably less decomposed than the

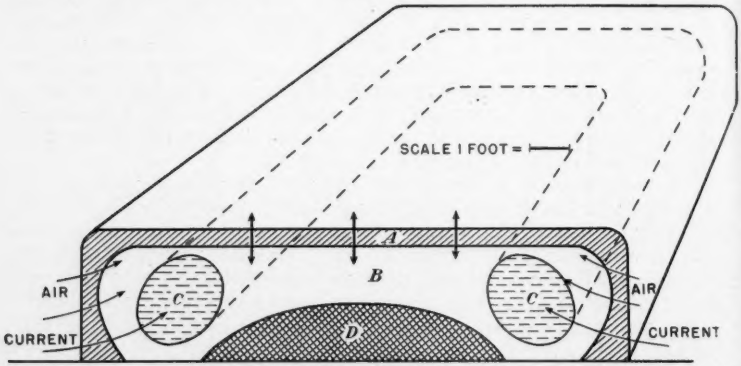

FIGURE 1.—Diagrammatic section through a mushroom compost heap, showing the usual gross differences in temperature and aeration in four areas: *A*, Aerobic, 110° F. or less; *B*, aerobic, 110° to 140°; *C*, aerobic, 150° to 180°; *D*, anaerobic, 100° to 130°.

remainder of the heap. Moreover, it has an unpleasant putrid odor and an acid reaction, whereas the remainder of the heap is usually alkaline. The compost in the high-temperature region (area *C*) soon attains a characteristic glossy brown color and has a caramel odor quite distinct from that of the manure in the remainder of the heap. In area *B* there is more evidence of fungus and actinomycete growth than in either area *C* or *D*. In the portions of area *B* with a temperature range between 130° and 140° F. the manure becomes speckled with a white spore dust from the extensive development of thermophilic actinomycetes. This actinomycete flora also appears in the beds during "sweating out," when similar conditions of aeration and temperature are encountered.

These characteristic differences in the appearance of the compost in various parts of mushroom compost heaps suggest differences in suitability for the growth of mushroom mycelium. The studies outlined in this paper were conducted primarily to learn whether there are demonstrable differences in this respect, (1) when conditions in the different areas are considered separately, and (2) when these conditions are considered in different combinations and sequences. Experiments are also described that were made to determine the effect of different controlled composting conditions on the rate of decomposition of the manure, and on the rate at which fresh manure is converted to compost suitable for the growth of mushroom mycelium.

EFFECT OF CONDITIONS IN DIFFERENT AREAS IN THE COMPOST HEAP ON SUITABILITY OF COMPOST FOR MUSHROOM MYCELIUM

The most direct method of studying the effect of conditions in different areas of the compost heap appeared to be the sampling of the various areas during successive stages of the composting process and the inoculation of the samples with mushroom spawn.

For purposes of comparison, triplicate samples of manure were taken separately from areas *B*, *C*, and *D* of a 20-ton compost heap at the time of mixing or "turning." Since the compost heap was

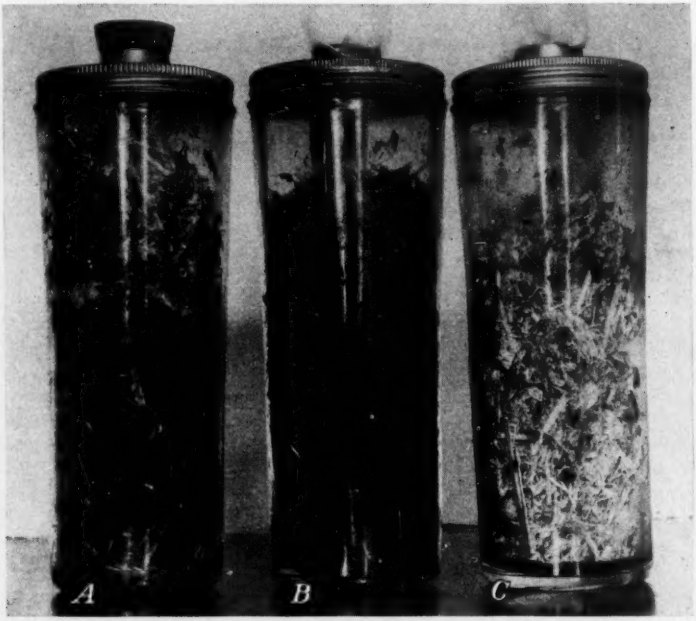

FIGURE 2.—Samples of manure incubated for 16 days under controlled conditions: *A*, 130° F., partly aerated; *B*, 170°, aerated; *C*, 130°, aerated. During incubation the jars were laid in a horizontal position to provide drainage, and the cotton plugs were removed for better aeration.

turned at weekly intervals, the manure had been subjected to composting conditions characteristic of the area sampled for at least 1 week before sampling. After the first week the manure in each area had been mixed with manure from other areas by one less turning than the number of weeks indicated in table 1. These samples were adjusted to about 160-percent moisture on a dry-weight basis, placed in quart jars, as shown in figure 2, and incubated in a horizontal position at 130° F. for 6 hours to kill insects. They were then cooled to 70° and inoculated with mushroom spawn.

A comparison of the growth of mushroom mycelium and of weed molds in these inoculated samples is shown in table 1. The samples

from area *B* (fig. 1) were well suited for the growth of mushroom spawn. The samples from areas *C* and *D*, however, were frequently overgrown with weed molds such as *Chaetomium*, *Coprinus*, *Trichoderma*, *Monilia*, and *Oedocephalum*. As a result, the growth of the mushroom mycelium was retarded or checked entirely. This type of experiment was repeated several times with essentially the same results.

TABLE 1.—*Comparative growth of mushroom spawn and weed molds in samples taken from different parts of a standard mushroom compost heap during successive turnings, when subjected to a 6-hour period of "sweating out" at 130° F., aerated, and subsequently spawned and incubated at 70°*

Area of compost heap sampled (fig. 1)	Outdoor-composting period	Average pH value at time of spawning	Growth [1] of mushroom spawn	Growth [1] of weed molds				
				Coprinus	*Trichoderma*	*Monilia*	*Chaetomium*	*Oedocephalum*
	Weeks							
B	1	8.1	++++	+	0	0	0	0
	2	7.8	++++	0	0	0	0	0
	3	7.7	++++	0	0	+++	0	0
	4	7.6	++++	0	0	0	0	0
C	1	6.8	+	+++	0	+++	0	0
	2	6.7	++++	0	++	0	++	++
	3	8.2	+++	0	0	++	0	0
	4	7.9	++	0	0	+++	0	0
D	1	8.2	++	++	+++	0	0	0
	2	8.7	++	+++	0	++	++	0
	3	8.6	++	+++	0	0	+++	0
	4	8.0	+++	0	0	++	0	0

[1] ++++=excellent; +++=good; ++=medium; +=poor; 0=no growth.

ALTERNATING COMPOSTING CONDITIONS

The fact that samples taken from area *B* were favorable for mushroom mycelium during the second and subsequent turnings, in spite of a probable admixture of compost formerly in areas *C* and *D*, suggested that conditions in area *B* tend to correct the harmful effects of anaerobic composting or of composting at temperatures over 150° F. To test this hypothesis, a series of samples was selected and prepared in the same manner as previously described except that before spawning the samples were composted in an incubator at 130° for 8 days instead of 6 hours.

In this experiment, as shown in table 2, nearly all the samples were well suited for the subsequent growth of mushroom mycelium and, with the exception of *Chaetomium* in a few samples, the weed molds were eliminated.

It was apparent that aerobic fermentation at 130° F. not only was favorable for subsequent growth of mushroom mycelium but had a beneficial effect on the compost that previously had been made unfavorable by anaerobic fermentation or by overheating. Other experiments were made in which it was repeatedly shown that, conversely, compost favorable for mushroom mycelium becomes unfavorable when incubated in glass jars for 1 or 2 days at an excessively high temperature or when incubated under anaerobic conditions even at a favorable temperature (130°). The injurious effects of overheating seemed to begin at about 150°. Compost so treated could again be made suitable by subjecting it to aerated fermentation for several days at 130°.

TABLE 2.—*Comparative growth of mushroom spawn and weed molds in samples taken from different parts of a standard mushroom compost heap during successive turnings, when subjected to 8 days of "sweating out" at 130° F., aerated, and subsequently spawned and incubated at 70°*

Area of compost heap sampled (fig. 1)	Outdoor-composting period	Average pH value at time of spawning	Growth [1] of mushroom spawn	Growth [1] of weed molds				
				Coprinus	*Trichoderma*	*Monilia*	*Chaetomium*	*Oedocephalum*
	Weeks							
B	1	8.1	++++	0	0	0	0	0
	2	7.9	++++	0	0	0	0	0
	3	8.0	++++	0	0	0	0	0
	4	7.8	++++	0	0	0	0	0
C	1	7.5	+	0	0	0	+++	0
	2	7.8	++++	0	0	0	0	0
	3	7.6	+++	0	0	0	0	0
	4	7.9	++++	0	0	0	0	0
D	1	8.4	+++	0	0	0	+	0
	2	8.4	+++	0	0	0	+++	0
	3	8.0	+++	0	0	0	0	0
	4	7.7	++++	0	0	0	0	0

[1] ++++=excellent; +++=good; ++=medium; +=poor; 0=no growth.

As a whole, these experiments indicate that if manure is first subjected to one set of composting conditions and then changed over to another it soon begins to assume characteristics typical of compost held in the new environment. This is probably explainable on the basis of the new environment's favoring a new fermentation flora, which in turn alters the composition of the compost so as to pave the way either for the mushroom mycelium or for weed molds as the case may be. The favorable flora in the case of the compost fermented under aerobic conditions at 130° F. appeared to be composed largely of thermophilic actinomycetes.

RATE OF DECOMPOSITION

Another point of interest relating to the different "areas" in a compost heap is the effect of aeration and temperature on the rate of decomposition of the manure. Numerous investigations have shown that organic matter decomposes more slowly under anaerobic than under aerobic conditions.[7] Recently Waksman, Cordon, and Hulpoi [8] have shown that stable manure compost incubated at 167° F. also decomposes much more slowly than at lower temperatures (84°, 122°, and 150°).

In order to obtain further information on this question, a series of 100 samples of fresh stable manure was incubated in glass jars (fig. 2) under different controlled conditions of temperature and aeration. At intervals of 5 days 2 jars were removed from each group of samples for dry-weight determination. The results of aerobic composting are given in figure 3. At temperatures ranging from 110° to 150° F. nearly half of the dry weight was lost during the first 20 days. At 170° there was very little loss. Conditions typical of area *D* (fig. 1) in compost heaps were simulated in a series of samples of manure composted in sealed jars which were incubated at 130°. This series lost less than 10 percent of its dry weight over a 30-day period.

[7] See footnote 3.
[8] See footnote 4.

The results of these experiments are in complete accord with earlier work in showing that both anaerobic conditions and temperatures approaching 170° F. are unfavorable for rapid decomposition of stable manure.

PREPARATION OF MUSHROOM COMPOST UNDER CONTROLLED FERMENTATION CONDITIONS

In the foregoing experiments, at the end of 2 weeks the manure held at 110° and 130° F. under aerated conditions appeared to be nearly as well decomposed as compost in the average mushroom bed. This suggested that fresh manure could be converted into compost suitable for mushroom mycelium in a comparatively short time under

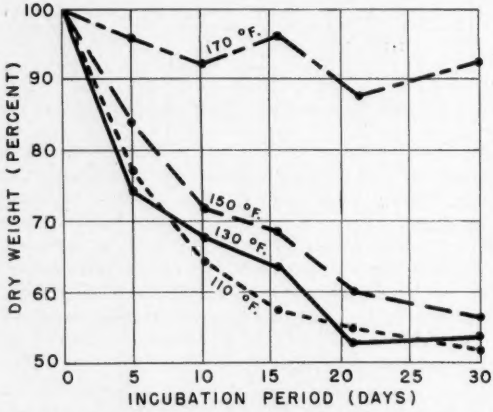

FIGURE 3.—Relation of temperature to rate of decomposition of stable manure under moist aerobic conditions.

favorable conditions. The excellent run of spawn that developed in some of the samples taken from area *B* after 1 week of outdoor composting, as shown in table 1, was further evidence pointing in this direction. Following up these leads, experiments were planned to learn something of the rate at which fresh horse manure could be converted to suitable mushroom compost under different controlled composting conditions.

In one series a comparison was made between manure alone, a mixture of manure and one-third soil by weight, and a mixture of manure and 2 percent of superphosphate. In all cases the supplementary material was thoroughly mixed with the manure, which was chopped into pieces less than 1 inch long and moistened to contain about 200 percent of water on a dry-weight basis. The type of jar used has already been illustrated (fig. 2). Moisture was maintained by inspection and rewatering every 3 days. Temperature was controlled within a 3-degree limit of variation at 110°, 120°, and 130° F. After fermentation periods of 9 days and 11 days, the bottles were removed from the incubator, cooled to room temperature, and spawned with mushroom mycelium.

A satisfactory growth of spawn was obtained in compost prepared at all three temperatures for both 9 days and 11 days. Spawn growth was dense and complete in the bottles containing the supplementary materials, but the manure alone became too wet and failed to support a good growth of spawn.

Another series of bottles containing a mixture of two-thirds manure, one-third soil, and 2 percent superphosphate was composted at 130° F. for 4 days, 6 days, 8 days, and 10 days before spawning. Mushroom mycelium developed satisfactorily in the manure composted 8 days and 10 days, but the samples composted for only 4 days and 6 days were soon overgrown with weed molds.

It was apparent from these experiments that under favorable fermentation conditions fresh manure can be converted into compost suitable for mushroom mycelium in about one-third the time required in the conventional outdoor compost heap. Subsequent experiments, not yet reported in detail, showed that compost prepared in this manner also is capable of producing a normal yield of mushrooms.

DISCUSSION AND CONCLUSIONS

It is apparent from the foregoing experiments that the usual method of composting manure for mushroom culture in outdoor heaps is not an efficient procedure. Only a limited portion of the heap, area *B* and parts of area *A* (fig. 1), is suitable for rapid decomposition and conversion of fresh manure to a satisfactory mushroom compost. On the other hand, a considerable portion of the conventional heap, areas *C* and *D*, is known to be decomposing at a comparatively slow rate and frequently to be in a condition favorable for subsequent growth of "weed molds" and unfavorable for mushroom mycelium. This is especially true during the first 2 weeks of composting, when all of the compost in the anaerobic and high-temperature areas is more or less unfavorable for mushroom mycelium.

As the composting proceeds the unfavorable conditions are less in evidence; area *C* becomes less extensive, owing to the disappearance of easily oxidized carbonaceous material, and the compost in area *D* becomes less acid with each turning. During outdoor composting a large part of the beneficial effect of continued composting probably comes from subjecting more and more of the compost to fermentation conditions in area *B* as a result of forking over and mixing the manure. In most cases the final conversion of unfavorable compost to a condition favorable to mushroom mycelium undoubtedly takes place during the "sweating out" process in the mushroom bed.

The sweating-out period is the only part of the composting process during which nearly all of the manure is fermenting under favorable conditions. Since at this time the compost is undergoing aerobic fermentation with a moisture content of 150 to 200 percent on a dry-weight basis and within a temperature range between 120° and 140° F., one can hardly escape the conclusion that the sweating-out process should be considered as an integral part of the composting procedure and not merely as a means of assisting in the eradication of insects and disease fungi. Outdoor composting might well be considered merely as a means of mixing and moistening the manure while carrying it through the initial explosive fermentation preparatory to a final favorable fermentation during sweating out.

Interesting practical considerations are suggested by this viewpoint and by the fact that suitable mushroom compost can be prepared from fresh manure in less than 2 weeks under conditions usually prevailing during sweating out. Small-scale experiments suggest that the outdoor composting period may be materially shortened or eliminated altogether provided sweating out is prolonged at the rate of 1 additional day of sweating out for every 3 days omitted from the usual outdoor composting. The extent to which it would be feasible or desirable to thus modify composting procedure in commercial practice will depend on the feasibility of controlling moisture and temperature during prolonged sweating out and on the prospects for saving labor or manure without reducing the yield of mushrooms.

SUMMARY

Consideration is given to the effect of physical factors, principally temperature and aeration, during composting on the subsequent growth of mushroom mycelium in the compost.

The suitability of compost for the growth of mushroom mycelium was determined by inoculation of selected samples with mushroom spawn and incubation at 70° F. in competition with the weed molds present in the compost.

Under aerobic conditions fermentation at temperatures between 120° and 140° F. for 8 to 10 days produced suitable compost. Temperatures over 150° for a few hours rendered the compost unsuitable. Anaerobic fermentation also produced unsuitable compost, even at favorable temperatures.

When manure was composted under different combinations and sequences of these conditions it tended to assume characteristics typical of the last environment to which it was subjected. Thus, aerobic fermentation at moderately high temperatures would again render compost suitable even though it had previously been made unsuitable by excessively high temperature fermentation or by anaerobic conditions.

In commercial mushroom growing, sweating out must be considered an integral part of the composting process. Close control of composting conditions is more important during sweating out than during outdoor composting, since conditions prevailing during sweating out constitute the last composting environment to which the manure is subjected before spawning.

USE OF LEMNA FOR NUTRITION STUDIES ON GREEN PLANTS[1]

By ROBERT A. STEINBERG

Associate physiologist, Division of Tobacco Investigations, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

The use of *Lemna minor* L. in a micromethod for study of the nutritional requirements of green plants has several obvious advantages. Equipment may be on a very small scale and selected for precision control of environmental factors at a small fraction of the initial cost and maintenance expense required for plants of ordinary size. Duration of experiments may be decreased to 14 days. Growth under aseptic conditions is entirely practicable and easily obtained, thus eliminating all possibility of erroneous results due to the presence of extraneous organisms. Precision control of environment should permit exact quantitative work capable of duplication elsewhere and in the future. Results obtained through the use of a micromethod may be directly checked with different crop plants, with consequent savings in the time and cost associated with large-scale exploratory work ordinarily necessary.

Trace-element studies with a green plant possess an obvious advantage over those with fungi. The former utilize carbon as carbon dioxide, which may easily be supplied free of trace-element impurities. The latter require a supply of sugar. Since a culture of *Aspergillus niger* Van Tiegh. contains 2.5 gm. of sucrose and 0.125 gm. of inorganic salts, it is evident that elimination of impurities in the carbon source is a very important consideration. The purest sugar obtainable contained 0.00087 percent of ash, or the equivalent of 435 parts per billion of impurities in the nutrient solution. This quantity compares with the 790 parts per billion whose addition is required for maximum growth of *Aspergillus*.

Lemna has been grown on synthetic nutrient solutions by many investigators (*2*, *3*)[2] and its mineral requirements shown to be quite similar to those of other green plants. The elements known to be required for its nutrition include carbon, hydrogen, oxygen, nitrogen, phosphorus, potassium, magnesium, sulfur, calcium, iron, and manganese (*3*). No positive evidence has been obtained, however, respecting its need for zinc, copper (*4*), molybdenum (*1*), gallium (*7*), and boron.

Aside from establishing conditions permitting maximum growth, and determining the accuracy in duplication of data, the ascertainment of the trace-element requirements of *Lemna* seemed most important. The higher plants require iron, zinc, copper, manganese, molybdenum, and boron, whereas *Lemna*, as mentioned, is only known to require iron and manganese. It was necessary, therefore,

[1] Received for publication November 4, 1940.

[2] Italic numbers in parentheses refer to Literature Cited, p. 430.

to adapt the method of nutrient-solution purification with calcium carbonate, employed with fungi (*5*, *6*) for the removal of trace-element impurities, to work with *Lemna*. Arnon and Stout (*1*, *8*) applied this technique to the purification of the individual salts of the nutrient solution in establishing the necessity for molybdenum by the tomato plant. Purification of the nutrient solution as a whole, in the manner employed with fungi, has certain advantages, particularly with a plant like *Lemna*, and was therefore used in this investigation. It was also believed that the use of nutrient-solution purification with *Lemna* would permit a closer comparison of the trace-element requirements of these two types of plants.

MATERIAL AND METHODS

An aseptic culture of *Lemna minor* obtained from Dr. A. Saeger, Junior College of Kansas City, Kansas City, Mo., was employed in these studies. The plants were grown on 50-cc. portions of nutrient solution in 200-cc. pyrex Erlenmeyer flasks provided with loose cotton plugs to maintain aseptic conditions. Reagent chemicals and water redistilled in a quartz still were used in the preparation of the nutrient solution. The flasks were sterilized after filling at 15 pounds pressure for 20 minutes. Transplantation of plantlets from the stock culture to the experimental flasks was with a platinum loop, employing the standard bacteriological technique to prevent contamination. For this purpose plantlets of minimum size having short roots were selected whenever possible. When harvested they were filtered off by means of a fritted-glass crucible (1 G 3), dried for 3 to 4 hours at 100 to 105° C., and weighed with the analytical balance when cool.

The plants were grown under different conditions. Initially an installation providing continuous light of 500 foot-candles with tungsten lamps (400 watts total) and a temperature of $25 \pm 1°$ C. was used. The growth period was 2 weeks. In this equipment the light was filtered through a 1-inch layer of water to minimize heating, and the cabinet was ventilated by means of a stream of chilled compressed air. When it was too hot to employ this installation, the flasks were placed in a large north window for 30 days. The illumination varied between 100 to 200 foot-candles. Later an electric refrigerator (9.2 cubic feet) having a special thermostat was provided with 500 foot-candles of white fluorescent light (100 watts total). Temperature in this cabinet averaged $25 \pm 0.5°$ C. Growth appeared normal under these conditions (continuous light) for the 2-week duration of an experiment. No ventilation was provided other than that brought about by opening the door of the refrigerator to examine the plants.

Where nutrient-solution purification was employed, calcium carbonate was added to a solution containing an excess of constituents. After steaming for 20 minutes at 100° C., the flask was set aside for 2 days. The solution was vigorously mixed by quick rotations of the flask at irregular intervals during this time. It was then filtered through a fritted-glass crucible (1 G 4) and made up to proper acidity with dilute (1.38 N) hydrochloric acid. The acid was redistilled for this purpose by placing two open pyrex bottles, one filled with concentrated acid, the other with water, in a sealed desiccator.

The purpose of these procedures was to permit the precipitate of dicalcium phosphate formed under these conditions to reach equilibrium, particularly as respects atmospheric carbon dioxide. When

employed with fungi, sufficient residual phosphate remains in solution because of the presence of fairly high concentrations of nitrogen salts and sugar. In the absence of sugar and high salt content, practically no phosphate may be left in the nutrient solution. When in equilibrium with atmospheric carbon dioxide, sufficient phosphate remains in solution to provide for the growth of 100 mg. of dry weight per 50 cc. with *Lemna*. In the case of larger plants requiring still higher quantities of phosphate, sufficient should be brought into solution by bubbling through gaseous carbon dioxide before filtering. Sufficient calcium remains in solution after purification, so that none need be added subsequently to filtration.

TRACE-ELEMENT DEFICIENCIES WITH REAGENT CHEMICALS

The experimental data of table 1 were obtained with several considerations in mind. Of these the foremost was to ascertain the precision with which results could be duplicated. Comparison of duplicate experiments 1 and 2 indicate that variations of ±10 percent from average may occur in percentage yields in simultaneous experiments. The data of duplicate experiments 3 and 4 show a somewhat lesser variation. In the first pair of duplicate experiments, only the results on molybdenum deficiency differ by not more than ±2.5 percent. In the second pair, with about double the salt content, agreement in results to less than ±5 percent occurred with deficiencies in iron, manganese, calcium, and boron. It is evident that differences of ±10 percent from average cannot be accepted as evidence for deficiency unless consistently reproducible at will.

Individual differences among the plants selected for initial transfer are probably responsible for a part of these variations. These can be minimized, if necessary, by the use of a larger number of plants. An equally important consideration is the proper adjustment of the components of the nutrient solution, since fluctuations in an unsuspected essential element can also result in large fluctuations in growth. Further study will be necessary to improve precision in results, but no serious difficulty is anticipated in attaining this objective.

TABLE 1.—*Growth of Lemna minor for 2 weeks under continuous illumination of 500 foot-candles of tungsten light at 25° C.*

Element omitted [1]	Experiment 1, weaker solution, [2] pH=4.70					Experiment 2, weaker solution, pH=4.70					Experiment 3, stronger solution, [3] pH=4.78				
	Yield	Proportion of maximum yield	Acidity at harvest	Size of plants [4]	Color [5]	Yield	Proportion of maximum yield	Acidity at harvest	Size of plants [4]	Color [5]	Yield	Proportion of maximum yield	Acidity at harvest	Size of plants [4]	Color [5]
	Mg.	*Pct.*	*pH*			*Mg.*	*Pct.*	*pH*			*Mg.*	*Pct.*	*pH*		
None	21.8	100.0	7.19	M.	4	24.2	100.0	7.33	M.	4	29.0	100.0	7.02	M.	4
Fe	7.5	34.4	6.75	M.	3	5.4	22.3	6.45	M.	2	7.3	25.2	6.42	M.	2
Zn	26.7	122.5	7.22	M.	4	25.7	106.2	7.20	M.	4	27.0	93.1	6.99	M.	3
Mn	2.5	11.5	6.11	S.	2	8.6	35.5	6.71	S.	2	11.2	38.6	6.42	S.	1
Mo	23.4	107.3	7.04	M.	4	27.3	112.8	7.25	M.	4	27.4	94.5	6.86	M.	4
Ga															
Ca	.9	4.1	5.94	S.	0	.5	2.1	5.95	S.	0	.6	2.1	5.60	0	0
B	22.2	101.8	7.15	M.	4	21.9	90.5	7.18	M.	4	27.1	93.4	6.89	M.	4

See footnotes at end of table.

TABLE 1.—*Growth of Lemna minor for 2 weeks under continuous illumination of 500 foot-candles of tungsten light at 25° C.*—Continued

Element omitted [1]	Experiment 4, stronger solution, pH=4.78					Experiment 5, stronger solution with 0.5 percent of sucrose, pH=4.35					Experiment 6, stronger solution with 0.5 percent of sucrose, pH=4.54				
	Yield	Proportion of maximum yield	Acidity at harvest	Size of plants [4]	Color [5]	Yield	Proportion of maximum yield	Acidity at harvest	Size of plants [4]	Color [5]	Yield	Proportion of maximum yield	Acidity at harvest	Size of plants [4]	Color [5]
	Mg.	*Pct.*	*pH*			*Mg.*	*Pct.*	*pH*			*Mg.*	*Pct.*	*pH*		
None	26.8	100.0	7.08	M.	4	74.9	100.0	6.97	M.	4	56.9	100.0	6.65	M.	4
Fe	7.8	29.1	6.38	M.	2	31.4	41.9	6.59	M.	2	32.5	57.1	6.41	L.	2
Zn	29.2	109.0	6.82	M.	3	74.8	99.9	6.10	M.	4+	54.0	94.9	5.88	M.	5
Mn	11.2	41.8	6.37	S.	1	20.0	26.7	5.97	S.	2	.9	1.6	4.62	S.	1
Mo	26.9	100.4	7.05	M.	4	61.8	82.5	6.27	M.	4+	50.5	88.8	6.22	M.	4
Ga											55.8	98.1	6.69	M.	4
Ca	.6	2.2	5.33		0	.7	.9	4.64		0	.2	.4	4.73		0
B	25.2	94.0	6.89	M.	4	70.5	94.1	6.83	M.	4	57.6	101.2	6.57	M.	4

[1] Iron, zinc, manganese, molybdenum, gallium, calcium, and boron at concentrations of 0.15, 0.04, 0.05, 0.02, 5.0, and 0.05 mg. per liter, respectively.
[2] Water, 1,000 cc.; KNO_3, 0.18 gm.; KH_2PO_4, 0.10 gm.; $MgSO_4 \cdot 7H_2O$, 0.04 gm.
[3] Water, 1,000 cc.; KNO_3, 0.35 gm; KH_2PO_4, 0.20 gm.; $MgSO_4 \cdot 7H_2O$, 0.10 gm.
[4] Tiny, small, medium, and large are indicated by first letter only. Zero indicates that no growth took place, owing to rapid death of the plants.
[5] Color is graded as 0 (white) to 5 (normal green).

There can be little doubt that omission of iron, manganese, and calcium from the nutrient solution may result in decreased growth when reagent chemicals are employed. Use of the higher salt concentration in experiments 3 and 4 tended to diminish the necessity for addition of these trace elements and to increase the uniformity in response.

In the last two duplicate experiments (5 and 6) the nutrient solution contained 5 gm. of sucrose per liter. The large increase in growth brought about through addition of sucrose definitely indicated photosynthesis to be the limiting factor for growth. This result was not unexpected, since Ashby and Oxley (*2*) had determined 1,600 foot-candles to be optimum for growth of *Lemna*. Miscellaneous experiments with increased carbon dioxide content of the air confirmed the need for additional light intensity. Results on trace-element deficiencies with 0.5 percent of sucrose were somewhat poorer with iron, while addition of molybdenum became necessary. Gallium became nontoxic, though in previous experiments its addition had brought about slightly decreased growth.

TRACE-ELEMENT DEFICIENCIES AFTER NUTRIENT-SOLUTION PURIFICATION

Nutrient-solution purification with calcium carbonate led to increased trace-element deficiencies (table 2) with iron, zinc, manganese, molybdenum, gallium, and boron. Sufficient residual calcium remained in the nutrient solution after purification to provide completely for the needs of the plant. Omission of copper from the purified nutrient solution did not decrease growth.

TABLE 2.—*Growth of Lemna minor in solutions purified with calcium carbonate*

Element omitted [1]	100–200 foot-candles of north light during July and August for 30 days										500 foot-candles of continuous white fluorescent light at 25° C. for 2 weeks														
	Purified with $CaCO_3$[2], p*H*=3.85					Purified with $CaCO_3$, p*H*=5.34					Purified with $CaCO_3$, p*H*=4.60					Purified with $CaCO_3$[3], p*H*=5.45					Purified with $CaCO_3$[4], p*H*=5.46				
	Yield	Proportion of maximum yield	Acidity at harvest	Size of plants [5]	Color [6]	Yield	Proportion of maximum yield	Acidity at harvest	Size of plants [5]	Color [6]	Yield	Porportion of maximum yield	Acidity at harvest	Size of plants [5]	Color [6]	Yield	Proportion of maximum yield	Acidity at harvest	Size of plants [5]	Color [6]	Yield	Proportion of maximum yield	Acidity at harvest	Size of plants [5]	Color [6]
	Mg.	*Pct.*	*pH*			*Mg.*	*Pct.*	*pH*			*Mg.*	*Pct.*	*pH*			*Mg.*	*Pct.*	*pH*			*Mg.*	*Pct.*	*pH*		
None	13.5	100.0	5.56	M.	5	17.3	100.0	5.94	L.	4	19.4	100.0	5.89	L.	4	7.9	100.0	5.73	M.	4	8.3	100.0	5.81	S.	3
Fe	6.1	.7	4.05	S.	0	2.6	15.0	5.42	S.	2	2.2	11.3	5.15	S.	4	1.0	12.7	5.48	T.	2	1.6	19.3	5.67	S.	1
Zn	4.5	33.3	4.19	M.	4	8.5	49.1	5.60	L.	4	17.7	91.2	5.48	L.	4	10.8	136.7	5.73	M.	4	8.2	98.8	5.83	M.	3
Mn	.3	2.2	3.83	T.	1	1.6	9.2	5.28	S.	5	1.4	7.2	4.63	T.	1	4.2	53.2	5.58	T.	2	5.3	63.9	5.74	S.	2
Mo	.6	4.4	3.79	S.	3	2.3	13.3	5.31	M.	4	11.1	57.2	5.47	L.	4	2.2	27.8	5.45	T.	3	2.4	28.9	5.65	M.	3
Ga	17.7	131.1	5.62	M.	5	9.2	53.2	5.71	L.	4	15.8	81.4	5.82	L.	4	4.6	58.2	5.57	S.	3	4.5	54.2	5.69	S.	1
B	6.8	50.4	5.06	M.	5	5.0	8.9	5.59	L.	4	14.6	75.3	5.77	L.	4	4.2	53.2	5.56	S.	4	6.0	72.3	5.76	M.	2

[1] Iron, zinc, manganese, molybdenum, gallium, and boron at 0.30, 0.04, 0.05, 0.02, 0.02, and 0.04 mg. per liter, respectively.
[2] Water, 1,000 cc.; KNO_3, 0.50 gm.; KH_2PO_4, 0.70 gm.; $MgSO_4.7H_2O$, 0.50 gm.; $CaCO_3$, 0.30 gm. HCl (1.38 N) added in quantities of 1.3 to 1.9 cc. per liter after filtration.
[3] Plants used in starting experiment much larger than usual.
[4] Plants used in starting experiment much larger than usual. The carbon dioxide content of the air was also increased.
[5] See footnote 4, table 1.
[6] See footnote 5, table 1.

SYMPTOMS OF TRACE-ELEMENT DEFICIENCY

Symptoms of trace-element deficiency were not specific but usually consisted of a general chlorosis, sometimes greater at the base of the frond and at other times at the apex. Extreme manganese deficiency led to a specific chlorosis characterized by the presence of irregular bleached spots.

Size of fronds usually decreased with deficiency except in the case of zinc. It seems probable that the differences in responses observed are correlated with specific differences in quantitative effect of the different elements and do not differ qualitatively.

RELATION OF ACIDITY TO RESULTS

Acidity ranging from pH 3.85 to pH 5.46 permitted growth of *Lemna*. This is about the range found favorable for growth of green plants in general. Growth appeared better at acidities less than that corresponding to pH 4.0.

The effect of growth on acidity of the nutrient solution was to cause a decrease. The extent of this decrease corresponded roughly to the extent of growth.

DISCUSSION

The results obtained with the calcium carbonate method of nutrient-solution purification indicate its applicability to work with green plants. Its use led to a definite improvement in the data on trace-element deficiencies with respect to the extent of the deficiencies and the number of elements needed. The necessity for the addition of iron, zinc, manganese, molybdenum, gallium, and boron to the nutrient solution was demonstrated following calcium carbonate purification.

The difficulties associated with the use of this method of studying the trace-element requirements of plants are due primarily to the complete precipitation of phosphate and possibly of certain of the essential trace elements. An attempt to employ this method before the discovery of the necessity for copper, manganese, molybdenum, gallium, and boron would most likely have resulted in such poor growth that the method would have been discarded. An adequate concentration of phosphate for *Lemna* can be assured by permitting the reaction mixture to reach equilibrium at room temperature before filtration. Though it was not necessary to resort to it with *Lemna*, the use of carbon dioxide at tensions higher than atmospheric should increase solution of the dicalcium phosphate precipitate according to known solubility data.

The results reported for gallium are not considered entirely adequate proof of its essentiality for *Lemna*. Gallium could not be replaced, however, by the other constituents of the nutrient solution, including nitrogen, phosphorus, potassium, magnesium, sulfur, calcium, iron, zinc, manganese, molybdenum, and boron. Neither were the following elements found in preliminary tests able to replace gallium in the nutrient solution: Copper, sodium, iodine, cobalt, lanthanum, scandium, vanadium, aluminum, germanium, tin, lead, titanium, zirconium, cerium, thorium, nickel, and beryllium. However, replacement tests with the elements have not been com-

pleted as yet. It seems quite probable, nevertheless, that gallium will be found essential for growth of *Lemna*, together with iron, zinc, copper, manganese, molybdenum, and boron (*7*).

An explanation for the improvement in results with boron would appear to require the assumption that only adsorption on the precipitate of dicalcium phosphate is concerned. No chemical reaction is known to the writer whereby boric acid would be precipitated under these conditions.

The results on trace-element requirements of *Lemna* parallel those of *Aspergillus* quite closely, both with respect to the elements that are essential and their effective concentrations. Exceptions exist, of course, since these forms differ widely in morphology and physiology. Boron and calcium are apparently not required by *Aspergillus*, whereas both are essential for *Lemna*. Zinc and copper are necessary for *Lemna*, in far lesser concentration than for *Aspergillus*, apparently. Results with *Lemna* were only fair with zinc, and the need for copper could not be demonstrated.[3]

Aspergillus requires iron, zinc, copper, manganese, molybdenum, and gallium in concentrations of 0.30, 0.30, 0.075, 0.075, 0.02, and 0.02 mg. per liter, respectively, after purification of the nutrient solution. *Lemna* required iron, zinc, copper, manganese, molybdenum, gallium, and boron in concentrations of 0.30, 0.04, 0.0, 0.05, 0.02, 0.02, and 0.04 mg. per liter, respectively, after nutrient-solution purification. It is interesting to note that the tomato plant was found by Arnon and Stout (*1*) to require 0.01 mg. of molybdenum per liter after calcium carbonate purification of the individual salts.

Further improvement in technique with *Lemna* should include modification in experimental conditions so as to use light of optimum intensity. This is about 1,600 foot-candles, as already mentioned. The basis for this statement is readily understood. The quantity of any trace-element impurity can be decreased by purification to an extremely low level, but, unless other conditions for growth are optimum, the poor yield of the control tends to eliminate this advantage. Poor yields of the control also increase the percentage error of duplication. It seems probable, therefore, that results on deficiencies obtained through application of this technique to crop plants should prove superior to those obtained with *Lemna*. *Lemna*, nevertheless, has proved adequate to serve in a micromethod for the study of mineral requirements of green plants.

SUMMARY

Lemna minor L. was grown aseptically in pyrex flasks with illumination supplied by tungsten lamps, fluorescent lamps, or north light. Deficiencies in iron, manganese, and calcium were found to exist in nutrient solutions prepared with reagent chemicals. Use of the calcium carbonate method of nutrient-solution purification led to a definite intensification of these effects. The addition of iron, zinc, manganese, molybdenum, gallium, and boron was found necessary after purification. The conditions necessary for the use of this method with green plants were established, and the use of the method is suggested in the study of trace-element requirements of crop plants.

[3] Later trials gave positive results with copper at 0.01 mg. per liter.

319107—41——4

LITERATURE CITED

(1) Arnon, D. I., and Stout, P. R.
1939. molybdenum as an essential element for higher plants. Plant Physiol. 14: 599–602, illus.

(2) Ashby, Eric, and Oxley, T. A.
1935. the interaction of factors in the growth of lemna. VI. an analysis of the influence of light intensity and temperature on the assimilation rate and the rate of frond multiplication. Ann. Bot. 49: [309]–336, illus.

(3) Hopkins, Edwin Fraser.
1934. manganese an essential element for green plants. N. Y. (Cornell) Agr. Expt. Sta. Mem. 151, 40 pp., illus.

(4) Saeger, Albert C.
1937. the concentration of copper in nutrient solutions for spirodela polyrrhiza. Amer. Jour. Bot. 24: 640–643, illus.

(5) Steinberg, Robert Aaron.
1919. a study of some factors in the chemical stimulation of the growth of aspergillus niger. Amer. Jour. Bot. 6: 330–372, illus.

(6) ———
1935. nutrient-solution purification for removal of heavy metals in deficiency investigations with aspergillus niger. Jour. Agr. Res. 51: 413–424.

(7) ———
1938. the essentiality of gallium to growth and reproduction of aspergillus niger. Jour. Agr. Res. 57: 569–574.

(8) Stout, P. R., and Arnon, D. I.
1939. experimental methods for the study of the role of copper, manganese, and zinc in the nutrition of higher plants. Amer. Jour. Bot. 26: 144–149.

BODY FORM IN GROWING CHICKENS[1]

By R. GEORGE JAAP

Associate professor in poultry husbandry, Oklahoma Agricultural Experiment Station

INTRODUCTION

Using adult female chickens fed and managed in a similar manner, Jaap and Thompson (*8*) [2] demonstrated heritable differences in body shape. The criteria of conformation used were the ratios obtained by dividing length of shank, body depth, and length of keel by the cube root of body weight. Such measures of body conformation in live birds have proved valuable for predicting visual body shape (*7*) when chickens or turkeys are being prepared for human consumption. The observations reported in earlier papers (*7*, *8*) were made in the latter part of the growth period and at maturity. The present study was planned to determine whether the above measurements may be used to detect heritable differences in body shape of live chickens at an early stage of growth, the so-called "broiler" ages.

A large proportion of market poultry is killed early in the growth period. In order to reserve for breeders those chickens having superior body conformation, it is important to measure body shape and other characteristics of market quality as early as 8 weeks after the chicks are hatched. In chicks of equal table quality, those whose body weights increase most rapidly during the first 8 to 12 weeks of life are generally considered the most profitable. More food is required for maintenance in the slower growing birds and turn-over of investment is not so rapid when growth is slow. These are probably some of the reasons why periodic increase in body weight is used in so large a number of studies as the measure of economic efficiency in the growth of chickens. It is evident that the most rapidly growing chickens do not, by virtue of their growth rate, necessarily have a body form that is pleasing to the eye.

METHOD OF SECURING DATA

Many investigators have demonstrated that season of hatch influences the growth rate of chickens. Kempster and Parker (*11*) have explained the differences in growth rate of their chicks as the result of retardation produced by high summer temperatures. Galpin (*6*), raising chickens under controlled temperatures, has suggested that the main factors influencing growth rate are vested in the egg, and these in turn, are probably related to factors controlling the physiological activity of the dam. Since seasonal differences might affect proportions as well as general body growth, the records used for this report were obtained for different kinds of chickens hatched at the same time and subjected to the same environment.

In order that the study might be applicable to the production of broiler and frying chickens in Oklahoma, 1,024 chicks were hatched

[1] Received for publication April 2, 1940.

[2] Italic numbers in parentheses refer to Literature Cited, p. 442.

on November 10, 1938. Classified according to parentage, these chicks belonged to five groups, produced by the following sires and dams:

Sire	*Dam*
Group 1, White Plymouth Rock	× White Plymouth Rock.
Group 2, Single-Comb White Leghorn	× Single-Comb White Leghorn.
Group 3, Dark Cornish	× White Plymouth Rock.
Group 4, Dark Cornish	× Single-Comb White Leghorn.
Group 5, White Plymouth Rock	× Single-Comb White Leghorn.

Hereafter reference to the different progeny groups is made by the breed name of the parents, as Plymouth Rock, Leghorn, Cornish × Plymouth Rock, Cornish × Leghorn, and Plymouth Rock × Leghorn.

To make the crossbreds directly comparable with the Standard varieties, the following procedure was used. An equal number of Cornish and Plymouth Rock males were alternated daily between two pens of Plymouth Rock females. These pens produced groups (1) and (3) given above. The crossbreds were distinguished by colored plumage and pea comb. Groups 4 and 5 were produced by daily alternating the respective males between two pens of Leghorn females. The crossbred chicks in this case were identified by the pea comb. This identification was checked at 8 weeks of age by the dominant, compact or close feathering characteristic of crosses involving the Cornish breed. The Leghorn chicks (group 2) were produced by alternating a number of males in pens of females whose breeding was similar to that of the females used for the production of groups 4 and 5. The Leghorn breed is not ordinarily considered desirable for the production of poultry meat, but because of a shortage of eggs from larger breeds in September and October, many commercial producers of chicks have been seeking information regarding the advisability of crossing large males with White Leghorn females. It was for this reason that the particular crosses to be included in these observations were chosen. The White Leghorn and White Plymouth Rock stock conform very closely to the breed standards as given by the American Poultry Association (*2*). The Dark Cornish used in these studies deviate from the Standard type by being somewhat longer legged and approaching the body carriage of the Game or Malay fowl.

To control environmental differences a proportionate number of each of the 5 groups was placed in each of 12 pens in a continuous floor brooding system. Thus each group as a whole was subjected to the same environment. For the first 4 weeks the chicks were fed an all-mash ration, No. CQ263.[3] At 4 weeks of age an additional feeder containing 2 parts cracked yellow corn, 1 part wheat, and 1 part kafir was placed in each pen. When the chickens reached 12 weeks of age, sex was recorded. At this time 400 of the living birds were retained for further observation and the remainder killed and plucked for visual observations on body form.

Individual body weights were recorded at hatching and every 2 weeks during the experiment. Shank length, anterior body depth, and keel length were measured at 8, 12, 16, and 20 weeks after hatching. Since previous unpublished records indicated that the female shank ceases increasing in length by about 4 months of age, shanks of

[3] PENQUITE, ROBERT, HELLER, V. G., and THOMPSON, R. B. RATIONS FOR THE PRODUCTION OF MEAT BIRDS, BROILERS, FRYERS AND ROOSTERS. Okla. Agr. Expt. Sta. Mim. Cir. 32, [4] pp., illus. 1939.

all females retained for study were measured every 2 weeks between 16 and 24 weeks of age.

METHODS OF SUMMARIZING DATA

The shape ratio

$$\frac{\text{Linear measurement}}{\sqrt[3]{\text{body weight}}} \tag{1}$$

is used to obtain a numerical expression of body form. While under certain conditions this ratio may be shown mathematically to be a function of the allometric or relative growth equation (*19*), it is used in this paper only as a simplified index of conformation. Lerner (*18, 19*) has criticized this ratio on the basis that it is in reality the initial growth index of Huxley and Teissier (*5*) and that it is a selected case of limiting allometry. Formula 1 presents no evidence as to how the shape of the chicken was attained. Since it has been shown that shank length exhibits positive allometry as compared with the body as a whole (*16*), the numerical value of the shape ratio when based on shank length would be expected to have different numerical values with increases in age of the bird. The value of the shape ratio as a means of predicting conformation differences was checked against a visual classification on those chickens that were killed and plucked at 12 weeks of age.

Shape ratios used in a previous report (*8*) were calculated for each linear measurement of each female. In the present data the shape ratios were obtained from means. As a test of validity of this procedure data for the Plymouth Rock females included in this report were calculated by both methods. The slight difference between the mean of ratios and the ratio of the means proved to be statistically nonsignificant. Analyses of variance were made from the original measurements. From the statistics thus obtained and the mean body weights of the groups being compared probability points were estimated.

To measure the relative growth of the part as compared with the body as a whole, a growth ratio was obtained from the equation

$$\frac{ln\ L_2 - ln\ L_1}{ln\ W_2 - ln\ W_1} \tag{2}$$

in which W_1 and L_1 are mean body weight and mean linear measurement, respectively, at the beginning, and W_2 and L_2 mean weight and mean measurement of the same group at the end of the growth period under consideration. Since these growth ratios are based on mean measurements from relatively large numbers of individuals, they probably present a fairly accurate estimate of relative growth in each group during the period of consideration.

Formula 2 represents one method of estimating the value of α in the allometric equation (*5*), $y=bx^{\alpha}$, when y is a part, x the standard or whole, and b the value of y when $x=1$. Many investigators have stressed that more nearly precise approximations of a constant growth ratio (α) are obtained from least-squares solutions which are based upon a number of points during growth. Solutions of formula 2 must not be confused with actual equilibrium constants for early

phases of growth. They are used simply to portray the relative growth of the part during the period under consideration and for comparison with changes in the shape ratio (formula 1) calculated at the beginning and end of the period. An expedient tool in practice is one which permits calculation at a given point in time.

SUMMARIZED DATA

CESSATION OF GROWTH IN LENGTH OF SHANK

Table 1 contains the mean shank length of 8 groups of females at 2-week intervals between 16 and 24 weeks after hatching. It may be observed that the shank in each group, except the Light Brahmas, reached its maximum length by 16 to 18 weeks. Shank length of the Light Brahmas increased up to 22 weeks of age. In all other groups the small numerical differences in shank length at successive periods after 18 weeks are well within the error of measurement. In view of the observation of Waters (*27*) it is probable that the apparent retarded growth of shank in the Light Brahma may be the result of a general retardation associated with late sexual maturity. Since there is very little information in the literature concerning the period of longitudinal shank growth further observations are in progress for both sexes.

The records presented in table 1 are in agreement with those of Latimer (*15*) for the tarsometatarsal length in White Leghorns. Latimer observed that the tarsometatarsus ceases to grow in length by 110 days after hatching in females and 142 days in males. Bone weight, however, increases to maturity. These findings would tend to invalidate the assumption of Lerner (*16*) that length (L) and weight (W) of the bird's tarsometatarsus bear a consistent relation as expressed by $W=CL^3$.

TABLE 1.—*Mean shank length of growing female chickens of different breeds and crosses aged 16 to 24 weeks*

Breed or cross of parents	Females	Shank length for indicated age (weeks)				
		16	18	20	22	24
	Number	*Centimeters*	*Centimeters*	*Centimeters*	*Centimeters*	*Centimeters*
Single-Comb Brown Leghorn	28	8.86	8.89	8.94	8.89	8.89
Rhode Island Red	14	10.36	10.57	10.64	10.59	10.59
Light Brahma	5	10.03	10.39	10.57	10.77	10.77
White Plymouth Rock	44	10.52	10.69	10.69	10.69	10.67
Dark Cornish male×White Plymouth Rock female	46	9.98	10.26	10.31	10.34	10.34
Single-Comb White Leghorn	37	9.47	9.63	9.55	9.60	9.58
Dark Cornish male×Single-Comb White Leghorn female	17	9.32	9.50	9.47	9.47	9.47
White Plymouth Rock male×Single-Comb White Leghorn female	36	10.06	10.19	10.16	10.13	10.19

Records on the increase in shank length with age of White Leghorns collected by Neunteufel (*25*) indicate that growth is very slow after 22 weeks. In Neunteufel's data the number of birds varied slightly with successive measurements, which may account for the discrepancy between her records and those presented in this paper. Lerner's data (*16, 17, 18*) indicate that shank length of both males and females

increased more rapidly than body weight in chickens between 16 and 20 weeks of age. The possibility of an earlier attainment of maximum shank length in more rapidly growing stock should be tested by further research.

Since the shank appears to reach its maximum length relatively early, normal increases in general body weight after this period will produce marked changes in a shank-body shape ratio (formula 1). The cessation of shank growth at an early age is not a phenomenon peculiar to chickens. Unpublished records of mean shank length of female turkeys show no increase between 24 and 52 weeks after hatching. Pålsson (*26*) observed that the cannon (carpal) bone of sheep reached its ultimate length by approximately 1 year but was still actively growing in thickness. Use of shank length in a shape ratio to determine whether hereditary conformation differences exist must, therefore, be at known ages.

Since Lumer (*20*) has demonstrated that the allometric equation is not valid when either of the variables approaches its upper asymptote, growth constants (*5*) for the relation between shank and body apply only during early phases of growth, at least prior to 16 weeks in females. The limiting equilibrium constant for adult proportions differs from the growth constant as a result of the normal growth of body after increases in shank length cease. Should the shank-body growth constant of Bantams be similar to that of normal sized breeds, a much lower limiting equilibrium constant is indicated from the data of Lerner (*16*).

Two factors which restrict length of the long bones to a greater degree than body weight have been studied by Landauer (*12, 13, 14*). From previous hatchability records the rather long-legged Cornish used in the present studies appeared not to carry the lethal factor (*13*) responsible for shortening of the long bones. Wright (*28*) has suggested special factors which control the growth of parts. Lumer (*21*) observed six allometric tribes in domestic dogs. Special factors such as these occurring in some strains and not in others may tend to depreciate the value of shank length in an expression of body form. In other words, anything which restricts the length of the long bones as compared with the remainder of the skeleton in certain strains would invalidate the use of shank length for comparing skeletal size of all strains of chickens.

GENERAL BODY GROWTH

The chickens used in this study were started on a diet [4] that has produced rapid early growth (table 2). Moreover, they were hatched at a season of the year that appears to be very favorable for rapid early increases in weight. As a result, growth rate of the body as a whole was greater than that ordinarily reported. The percentage growth rates calculated according to the arithmetical method of Brody (*3*) are presented in table 2. In males, percentage growth rate from 0–4 weeks of age ranges from 148.3 for the Cornish × Plymouth Rock cross to 153.4 for the Cornish × Leghorn cross. The pullet growth rates during the same period ranged from 143.9 for the Cornish × Plymouth Rock progeny to 150.0 percent for progeny of the Plymouth Rock × Leghorn cross. These growth rate figures are considerably higher than those given by Kempster and Parker (*11*) for

[4] See footnote 3.

early hatched pullets. Similarly, the growth rate of both sexes from 4 to 8 and 8 to 12 weeks may be considered above normal. When the logarithms of body weights at 2-week intervals are plotted against units of time, these data do not approach a straight line relation at any time during the period of observation to 18 weeks. Therefore, body weight in this study did not increase at a constant percentage rate.

TABLE 2.—*Percentage growth rates*[1] *of chickens used in this study while on the starting diet*

Breed or cross	Males	Growth rate for indicated age period (weeks)			Females	Growth rate for indicated age period (weeks)		
		0–4	4–8	8–12		0–4	4–8	8–12
Dark Cornish × White Plymouth Rock	*Number* 89	*Percent* 148.3	*Percent* 99.5	*Percent* 58.8	*Number* 105	*Percent* 143.9	*Percent* 95.9	*Percent* 54.6
White Plymouth Rock	164	150.2	101.1	58.9	151	148.2	95.8	54.0
White Plymouth Rock × Single-Comb White Leghorn	134	153.3	101.1	56.9	126	150.0	94.6	50.1
Dark Cornish × Single-Comb White Leghorn	25	153.4	100.3	52.9	44	147.9	93.6	50.7
Single-Comb White Leghorn	63	149.3	94.0	54.8	64	147.3	87.4	45.6

[1] Percentage rate of growth $= \frac{(W_2 - W_1)}{\frac{1}{2}(W_1 + W_2)} \times 100$, when W_1 is body weight at the beginning and W_2 weight at end of the period under consideration.

In each of the five groups, the male chicks weighed more than the females at 2 weeks of age. This mean difference between sexes within groups was highly significant statistically. Whether this was due to an early difference in the sex stimulus for growth remains to be determined. Although Jourdain (*9*) obtained more rapid growth in pullets raised separately from the cockerels, it is inconceivable to the author that the presence of male chicks in these groups could have influenced the growth rate of their sisters at such an early age. Munro and Kosin (*24*) have observed a sex difference in chick weight at hatching. Ackerson and Mussehl (*1*) found males to be heavier than females at 1 week of age. Under restricted diet and an integrated value for growth based on two lots hatched 1 day apart, Mstislavsky (*23*) [5] observed slightly superior growth of males during the first 15 days after hatching. This difference then disappeared and was not again observed until approximately the forty-fifth day. Studies are in progress to determine whether a ration which produces maximum early growth favors an early expression of the sex difference in growth rate when sexes are reared separately from hatching.

Mortality to 12 weeks of age was relatively low. In each of the Plymouth Rock and Plymouth Rock × Leghorn groups the loss was 6.5 percent. Mortality in Leghorns was 3.8 percent; in Cornish × Leghorn, 1.5 percent; and in Cornish × Plymouth Rock, 5.8 percent. Data for chicks that died are excluded from the summarized records.

RELATIVE GROWTH OF SHANK AND BODY

Table 3 presents the available material relative to changes in body shape which result from the relative rate of shank growth. Assuming

[5] The author is indebted to Dr. V. L. Maleev of the Engineering Division, Oklahoma Agricultural and Mechanical College, for interpretation of this part of the cited article.

that a growth ratio of 0.333 (*16*) represents equal growth rate of shank and body between 8 and 12 weeks after hatching, shank length increased more rapidly than body weight in all cases except Cornish × Leghorn and Cornish × Plymouth Rock females. By the method described previously it is estimated that the positive allometry of shank length observed in Leghorn females and all males between 8 and 12 weeks is statistically significant.

Lerner (*18*) has criticized the author's use of the shape ratio, stating that it is less precise than solutions based on logarithms. A very consistent relation may be noted between the value of the growth ratio (formula 2) and the increase in numerical value of the shape ratio (formula 1) for the 8- to 12-week period. The data shown in tables 3 and 4 clearly indicate that the differences in the shape ratios that are calculated arithmetically are as sensitive a measure of growth changes as the growth ratio calculated from the logarithms of the data at two points in the growth period. It is possible that this close association may be observed only when birds of the same age are being compared.

TABLE 3.—*Relative growth of shank length and body weight with resulting changes in body shape of chickens between 8 and 16 weeks of age*

Sex, breed, or cross	Shape ratio [1] at 8 weeks	Growth ratio [2] at 8–12 weeks	Shape ratio [1] at 12 weeks	Growth ratio [2] at 12–16 weeks	Shape ratio [1] at 16 weeks	Birds of superior shape at 12 weeks
Females:						*Percent*
Dark Cornish × White Plymouth Rock	587	0.336	588	0.265	575	79
White Plymouth Rock	591	.346	595	.265	585	71
Dark Cornish × Single-Comb White Leghorn	584	.334	585	.169	555	91
White Plymouth Rock × Single-Comb White Leghorn	598	.341	601	.248	585	58
Single-Comb White Leghorn	604	.372	615	.207	590	30
Males:						
Dark Cornish × White Plymouth Rock	599	.375	616	.341	618	61
White Plymouth Rock	613	.369	626	.370	636	48
Dark Cornish × Single-Comb White Leghorn	600	.366	611	.330	610	----------
White Plymouth Rock × Single-Comb White Leghorn	618	.359	628	.326	626	58
Single-Comb White Leghorn	624	.348	629	.339	630	42

[1] By formula 1, p. 433.
[2] By formula 2, p. 433.

The data presented here tend to support Lerner's assumption (*16*) that a value of 0.333 for the growth ratio represents relatively equal increases in shank length and body weight, i. e. isometry. In table 3 the column labeled "Birds of superior shape at 12 weeks" gives the percentage of those killed at 12 weeks of age that appeared superior in body conformation after feathers had been removed. A fair agreement between the shank-body shape ratio at 12 weeks and the visual grading may be observed within each sex. A decrease in the numerical value of the shape ratio is associated with a higher percentage attaining a superior rating on visual body shape.

Since the shank-growth ratio for females between 12 and 16 weeks of age is smaller than 0.333 it is apparent that the body grew more rapidly than the shank increased in length. The resulting changes in body form may be seen in the numerical reduction of the shank-body shape ratio at 16 weeks of age in females. During this same period growth rate of the male shank equaled or exceeded body growth;

however, a marked reduction may be observed in the relative increase in shank length between 12 and 16 weeks of all male groups, except the Plymouth Rock. Since these males were used to obtain other information complete records are not available at 20 weeks of age. The body weight of those males measured at 20 weeks had increased more rapidly than shank length. The data presented in table 3 show in a more marked degree similar fluctuations in relative growth values observed by Lerner (*17*) for individual birds. They do not invalidate the use of a constant growth ratio for early stages of growth but they do indicate that approximately 12 weeks of age may be the upper age limit for its use with females.

BODY FORM AS INFLUENCED BY RELATIVE CHANGES IN DEPTH

Body depth is here considered as the minimum distance between the anterior extremity of the keel and the dorsal surface of the body. The depth-body shape ratios and growth ratios calculated from formulas 1 and 2 are given in table 4. Between 8 and 12 weeks body depth increased relatively much more slowly than body weight. As a result the depth-body shape ratios are much smaller at 12 weeks. The body of all varieties and both sexes, therefore, became much plumper by 12 than they were at 8 weeks after hatching. After this period body depth and weight appeared to increase in about the same percentage relation. The majority of the differences between shape ratios at 12 and 16 weeks may be considered statistically nonsignificant.

TABLE 4.—*Relative growth of body depth and weight of chickens, and resulting changes in body shape*

Sex, breed, or cross	Shape ratio [1] at 8 weeks	Growth ratio [2] at 8–12 weeks	Shape ratio [1] at 12 weeks	Growth ratio [2] at 12–16 weeks	Shape ratio [1] at 16 weeks	Growth ratio [2] at 16–20 weeks	Shape ratio [1] at 20 weeks
Females:							
Dark Cornish × White Plymouth Rock	575	0.252	550	0.364	556	0.298	551
White Plymouth Rock	590	.266	568	.376	577	.348	579
Dark Cornish × Single-Comb White Leghorn	580	.226	549	.323	548	.247	536
White Plymouth Rock × Single-Comb White Leghorn	592	.225	560	.317	557	.332	557
Single-Comb White Leghorn	598	.157	552	.326	550	.308	547
Males:							
Dark Cornish × White Plymouth Rock	578	.272	558	.368	568	----------	----------
White Plymouth Rock	586	.291	571	.356	577	----------	----------
Dark Cornish × Single-Comb White Leghorn	578	.258	555	.345	558	----------	----------
White Plymouth Rock × Single-Comb White Leghorn	593	.260	568	.341	569	----------	----------
Single-Comb White Leghorn	597	.205	555	.324	553	----------	----------

[1] By Formula 1, p. 433.
[2] By Formula 2, p. 433.

CHANGES IN RELATIVE KEEL LENGTH

It has previously been shown (*7*) that changes in relative keel length do not materially affect the visual conformation of the body. Between 8 and 12 weeks of age there was very little change in proportional keel length, but between 12 and 16 weeks keels of both sexes in all groups became proportionally much longer. During this period the mean shape ratios of the females increased from 509 to

570 and the males from 551 to 571. These data indicate that the growth of the keel differed distinctly from that of shank length. Further evidence of an inherent difference between growth in length of keel and growth in length of the long bones is found in the data of Caskey and Norris (*4*). In these experiments a deficiency of manganese during the embryonic stages limited the postnatal growth of the long bones but did not influence the length of the keel. The close agreement between the differences in shape ratios at the beginning and end of each period and the growth ratios for that period was also observed in the data for keel length. Isometry, or relatively equal growth of the part to the whole in all three linear measurements (shank length, body depth, and keel length), appears to give a growth ratio of approximately 0.333.

HERITABLE SHAPE DIFFERENCES BETWEEN BREEDS AND CROSSES

It has been impossible by the use of weight, depth, shank and keel length to detect significant differences in body conformation at 8 weeks of age. Proportional differences in body dimensions are not very large at this age and variability in general is greater. By 12 weeks of age many of the differences in body shape are highly significant as estimated by probability points secured from the actual data. Differences between certain female groups exist in all three criteria. Male groups differed most in proportional body depth at 12 weeks. In both sexes, the Cornish male parent increased the relative body weight as compared with body depth and shank length. The relation between relative keel lengths of the various groups varied through the observation period. The only consistent group difference was the Cornish × Leghorn female progeny which had a relatively shorter keel length at 8, 12, 16, and 20 weeks of age.

Proportionally shallow bodies and short shanks indicate superior body shape of chickens after the feathers are removed. On the basis of these two criteria (tables 3 and 4) the visual body shape after killing and dressing would be in the following decreasing order of preference at 12 weeks of age. Males: (1) Cornish × Leghorn, (2) Cornish × Plymouth Rock, (3) Plymouth Rock × Leghorn and Plymouth Rock, and (4) Leghorn; females: (1) Cornish × Leghorn, (2) Cornish × Plymouth Rock, (3) Plymouth Rock, (4) Plymouth Rock × Leghorn, and (5) Leghorn. To test this relation further a random sample of each sex group, except Cornish × Leghorn males, was taken and the birds were graded into visual preference groups after they were killed and the feathers removed. The distribution of the birds into 4 arbitrary preference groups is shown in table 5. The plumpest birds were placed in conformation grade 1. Grades 2, 3, and 4 each had inferior shape quality. The guide used to determine shape quality was that given by the Bureau of Agricultural Economics [6]. The percent of the total of each sex group placed in grades 1 and 2 compares very favorably with the order of preference judged from live bird measurements. The relation is not as close in this population at 12 weeks of age as was found for mature cockerels and capons (*7*).

[6] U. S. DEPARTMENT OF AGRICULTURE, BUREAU OF AGRICULTURAL ECONOMICS. CLASSIFICATION AND TENTATIVE SPECIFICATIONS FOR U. S. STANDARDS AND GRADES FOR DRESSED CHICKEN. 8 pp. Washington, D. C. 1938. [Mimeographed.]

TABLE 5.—*Distribution of birds into 4 arbitrary preference grades according to the number in visual conformation grades after killing and plucking at 12 weeks, and also the percentage of birds in the upper 2 grades*

Breed or cross	Sex	Birds in conformation grade No.				Birds in grades Nos. 1 and 2
		1	2	3	4	
		Number	*Number*	*Number*	*Number*	*Percent*
Dark Cornish × White Plymouth Rock	Male	10	12	10	4	61
	Female	21	21	7	4	79
White Plymouth Rock	Male	14	40	40	19	48
	Female	31	36	19	8	71
White Plymouth Rock × Single-Comb White Leghorn	Male	12	34	23	10	58
	Female	17	29	27	7	58
Dark Cornish × Single-Comb White Leghorn	Female	12	9	1	1	91
Single-Comb White Leghorn	Male	1	9	8	6	42
	Female	1	6	14	2	30

From the analyses presented it may be observed that the criteria of body shape which have been used in this study roughly segregate the visual classes. A much greater refinement of measurements and technique is needed to obtain nonsubjective accurate records of body shape in chickens during their early growth period.

PERCENTAGE OF EDIBLE FLESH AND BODY CONFORMATION

To determine whether there is any relation between criteria of body conformation and percent of edible flesh, a number of males were deboned and examined. Plymouth Rock and Cornish × Plymouth Rock crossbred males were killed weekly as they reached approximately 6 pounds of live body weight. Another group consisting of Cornish × Leghorn and Plymouth Rock × Leghorn crossbred males were killed as they approached 5 pounds. Edible flesh is considered to include the boneless flesh and skin and the cleaned giblets. The mean shape ratios and mean percentages of edible flesh of dressed body weight are given in table 6. The Cornish parent has caused a statistically significant increase in edible flesh in both crosses. This is in agreement with the observation of Maw (*22*) for males at 26 weeks of age. The males of the present report ranged between 18 and 27 weeks of age at the time the data were collected. The differences in mean shank ratios correspond very closely to the differences in percentage of flesh of the groups. These differences are highly significant. Within each bird percentage of edible flesh and proportional shank length are not correlated. This may be attributable to a relatively high variability within groups and indicates that the shank-body shape ratio is not a satisfactory basis for predicting the percentage of flesh in individual birds. Jull, Quinn, and Godfrey (*10*) state that flesh production is apparently inherited largely independently of skeletal structure but present no evidence to support this statement in the case of the chicken.

Percentage of edible flesh is no staisfactory criterion of visual market quality. It appears that a chicken may attain superior body shape in two ways, namely, by an increase in flesh and fat and by changes in the basic shape of the skeleton. Thus far it has been impossible to differentiate birds which attain a plump body through additional flesh and those whose skeleton is so contsructed as to make

the body appear plump. It is indicated that the Cornish as a breed has both characteristics and apparently transmits them to its progeny. Actually the difference in percentage of edible flesh in these widely divergent groups ranges from only 1.5 to 1.9 percent of the dressed body weight. Stated in another manner, this mean difference in percent of edible flesh in 5-pound roasters may be expected to be less than one-tenth of a pound. Though this difference is highly significant statistically its value to the consumer is probably less than the value of a desirable body conformation. From the market standpoint it is important that the carcass appear plump regardless of ratio between bone and edible flesh.

TABLE 6.—*Mean shape ratios and mean percentage of edible flesh for male birds of different progeny groups when killed at approximately 5 or 6 pounds live weight*

Progeny group	Males	Mean dressed weight	Mean live-weight ratios			Ratio of edible flesh to dressed weight
			Depth	Shank	Keel	
	Number	*Pounds*				*Percent*
White Plymouth Rock	36	5.37	577	629	606	51.4
Dark Cornish × White Plymouth Rock	41	5.48	576	615	601	52.9
White Plymouth Rock × Single-Comb White Leghorn	20	4.34	586	632	614	51.4
Dark Cornish × Single-Comb White Leghorn	21	4.35	566	601	595	53.3

SUMMARY

A thousand and twenty-four chicks were hatched from two breeds and three crosses. These five groups were reared together in such a manner that an equal proportion of each group was subjected to such environmental variations as occur in a uniform brooding system. General body growth was considerably above normal. Male chicks were heavier than their sisters at 2 weeks after hatching.

Longitudinal increases in shank length were observed to cease by 16 to 18 weeks after hatching in all female groups except the Light Brahmas. From these observations it is apparent that the growth constant should be differentiated from that of the limiting equilibrium constant based on relative shank length at maturity. Since shank length rapidly approaches its upper asymptote in growth it is suggested that relative growth constants for the shank length of female chickens may apply only to growth prior to 12 weeks of age. Evidence that 16 weeks may be a comparable age in males is presented.

In this experiment anterior body depth became relatively smaller between 8 and 12 weeks after hatching in both sexes. Extreme differences between relative longitudinal growth of keel and shank were observed.

Heritable difference in conformation manifested between groups could not be readily distinguished until the birds were 12 weeks of age, a fact which demonstrates that a much greater refinement in methods of describing form is needed for growing chicks. The Dark Cornish male parents increased the percent of edible flesh in their crossbred male progeny. Since this increase amounted to only about 1.5 to 1.9 percent of the dressed body weight, body shape is considered more important than edible flesh in poultry practice. Percentage of edible flesh is not satisfactory as a criterion of visual conformation.

LITERATURE CITED

(1) Ackerson, C. W., and Mussehl, F. E.
1930. SEX DIFFERENCES IN THE NORMAL GROWTH RATE OF CHICKS. Jour. Agr. Res. 40: 863–866.
(2) American Poultry Association.
1938. THE AMERICAN STANDARD OF PERFECTION . . . 492 pp., illus. [Davenport, Iowa.]
(3) Brody, Samuel.
1927. GROWTH AND DEVELOPMENT WITH SPECIAL REFERENCE TO DOMESTIC ANIMALS. III. GROWTH RATES, THEIR EVALUATION AND SIGNIFICANCE. Mo. Agr. Expt. Res. Bul. 97, 70 pp., illus.
(4) Caskey, C. D., and Norris, L. C.
1940. MICROMELIA IN ADULT FOWL CAUSED BY MANGANESE DEFICIENCY DURING EMBRYONIC DEVELOPMENT. Soc. Expt. Biol. and Med. Proc. 44: 332–335, illus.
(5) Huxley, J. S., and Teissier, G.
1936. TERMINOLOGY OF RELATIVE GROWTH. Nature [London] 137: 780–781.
(6) Galpin, N.
1939. FACTORS AFFECTING THE POST-NATAL GROWTH OF BROWN LEGHORN CHICKENS. Empire Jour. Expt. Agr. 7: [139]–144, illus.
(7) Jaap, R. George, and Penquite, Robert.
1938. CRITERIA OF CONFORMATION IN MARKET POULTRY. Poultry Sci. 17: 425–430.
(8) ——— and Thompson, R. B.
1940. HERITABLE DIFFERENCES IN CONFORMATION OF ADULT FEMALE FOWL. Poultry Sci. 19: 73–78.
(9) Jourdain, W. L.
1936. COMPARISON OF GROWTH IN SEXED AND IN UNSEXED PULLETS. New Zeal. Jour. Agr. 52: 293–295.
(10) Jull, Morley, A., Quinn, Joseph P., and Godfrey, A. B.
1933. IS THERE AN EGG-LAYING TYPE OF THE DOMESTIC FOWL? Poultry Sci. 12: 153–162.
(11) Kempster, H. L., and Parker, Jesse E.
1936. THE NORMAL GROWTH OF CHICKENS UNDER NORMAL CONDITIONS. Mo. Agr. Expt. Sta. Res. Bul. 247, 46 pp., illus.
(12) Landauer, W[alter].
1934. STUDIES ON THE CREEPER FOWL. VI. SKELETAL GROWTH OF CREEPER CHICKENS, WITH SPECIAL REFERENCE TO GROWTH OF THE LONG BONES. Conn. (Storrs) Agr. Expt. Sta. Bul. 193, 79 pp., illus.
(13) ———
1935. A LETHAL MUTATION IN DARK CORNISH FOWL. PRELIMINARY REPORT. Jour. Genet. 31: [237]–242, illus.
(14) ———
1938. A LETHAL MUTATION IN JAPANESE BANTAM FOWL. (Abstract) Genetics 23: 155.
(15) Latimer, Homer B.
1927. POSTNATAL GROWTH OF THE CHICKEN SKELETON. Amer. Jour. Anat. 40: 1–57, illus.
(16) Lerner, I. Michael.
1937. RELATIVE GROWTH AND HEREDITARY SIZE LIMITATION IN THE DOMESTIC FOWL. Hilgardia 10: 511–560, illus.
(17) ———
1938. GROWTH RATIO OF THE FOWL'S TARSOMETATARSUS. I. VARIATION WITH RESPECT TO BODY WEIGHT. II. VARIATION WITH RESPECT TO TIME. Growth 2: [135]–144.
(18) ———
1939. ALLOMETRIC STUDIES OF POULTRY. 7th World's Poultry Cong., Cleveland, Proc. 1939: 85–88.
(19) ———
1939. PREDICTABILITY OF BODY WEIGHT FROM LIVE SHANK MEASUREMENTS. Poultry Sci. 18: 378–380.

(20) LUMER, HYMAN.
1937. THE CONSEQUENCES OF SIGMOID GROWTH FOR RELATIVE GROWTH FUNCTIONS. Growth 1: [140]–154, illus.
(21) ———
1940. EVOLUTIONARY ALLOMETRY IN THE SKELETON OF THE DOMESTICATED DOG. Amer. Nat. 74: 439–467, illus.
(22) MAW, W. A.
1933. MEAT PRODUCTION IN POULTRY. U. S. Egg & Poultry Mag. 39 (July): 18–47, illus.
(23) MSTISLAVSKY, M.
1939. [GROWTH OF WHITE LEGHORN CHICKS IN THE FIRST THREE MONTHS OF POST-EMBRYONIC DEVELOPMENT.] Moscow Univ. Mem. Uchenye Zapishi 20: [259]–327, illus. [In Russian. English summary, pp. [326]–327.
(24) MUNRO, S. S., and KOSIN, IGOR L.
1940. THE EXISTENCE OF A SEX DIFFERENCE IN THE WEIGHT OF DAY-OLD CHICKS, WITH FURTHER DATA ON THE EGG WEIGHT-CHICK WEIGHT RELATIONSHIP. Sci. Agr. 20: 586–591.
(25) NEUNTEUFEL, GERTRUDE.
1930. WACHSTUMSBEOBACHTUNGEN AM WEISSEN AMERIKANISCHEN LEGHORN. Kreisgeflügelzuchtanst. f. Oberbayern, Erding, Jubiläumsber. 1930: 33–65.
(26) PÁLSSON, H.
1940. MEAT QUALITIES IN THE SHEEP WITH SPECIAL REFERENCE TO SCOTTISH BREEDS AND CROSSES. I. Jour. Agr. Sci. [England] 29: [544]–626, illus.
(27) WATERS, N. F.
1937. BODY WEIGHT, EGG WEIGHT, SEXUAL MATURITY AND GROWTH RATE IN THE DOMESTIC FOWL. Poultry Sci. 16: 305–313, illus.
(28) WRIGHT, SEWALL.
1932. GENERAL, GROUP, AND SPECIAL SIZE FACTORS. Genetics 17: [603]–619, illus.

U. S. GOVERNMENT PRINTING OFFICE: 1941